

THESIS

BEE DIVERSITY AND ABUNDANCE UNDER A GRAZED COVER CROPPING MANAGEMENT SYSTEM  
IN EASTERN COLORADO AND SOUTHWESTERN NEBRASKA AND EVALUATING THE ROLE OF  
BEEKEEPING EDUCATION AND MANAGEMENT ON HONEY BEE HIVE OVERWINTERING SUCCESS  
IN COLORADO

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## ABSTRACT

### BEE DIVERSITY AND ABUNDANCE UNDER A GRAZED COVER CROPPING MANAGEMENT SYSTEM IN EASTERN COLORADO AND SOUTHWESTERN NEBRASKA AND EVALUATING THE ROLE OF BEEKEEPING EDUCATION AND MANAGEMENT ON HONEY BEE HIVE OVERWINTERING SUCCESS IN COLORADO

Bee pollination is essential to the production of many valuable crops in addition to facilitating the reproduction of non-crop flowering plants in the environment. Managed and wild populations of bees face unique and overlapping challenges. Wild bees have been negatively impacted by habitat and forage loss as a result of agricultural intensification. There has been headway in finding solutions that offset the environmental impact of agriculture that benefit wild bees without being a financial burden to the producer. Solutions often include the introduction or retainment of forage and habitat within the agricultural landscape. One example of this is the inclusion of bee-friendly cover crops into a crop rotation. Cover crops can promote agroecosystem services such as, nitrogen fixation, reduce erosion etc., and also provide nesting habitat and forage for pollinators. Chapter one explores bee diversity and abundance under a grazed cover cropping management system in eastern Colorado and southwestern Nebraska. Blue vane traps were used to conduct monthly collections of bees within three cover-cropped fields to evaluate diversity and abundance of bees under varying grazing conditions. There was higher diversity of bee genera in fields where grazing intensity was low but bee abundance was higher in grazed fields with the highest representation being from the ground-nesting genus, *Lasioglossum*. Setting aside some cover-cropped areas to

remain ungrazed, allowing plants to come into bloom will provide nutrition and nesting resources for bees in this region.

Pathogens and pests are another set of challenges that pollinators face in the environment. Managed bees can be a source of inoculum for wild bees if hives are not kept healthy. Managed bees often visit the same forage sites as wild bees. These communal areas where wild and managed bees interact present opportunities for pathogens to spill over from the managed populations to the wild populations. Pathogen development and spread within managed populations can often be prevented by good beekeeper practices that keep hives healthy. Chapter two explores the role that beekeeping education plays in honey bee hive health and survival among hobby beekeepers across Colorado. While most commercial pollination services are provided by professional beekeepers with 500 or more hives, the majority of beekeepers in the United States are backyard beekeepers with typical operations of fewer than 50 hives. Despite increased interest in backyard beekeeping, average hive loss in the United States is still 35%-40%. Hive survival depends on beekeeper intervention, but many backyard beekeepers lack training and are unfamiliar with the hive management techniques necessary for maintaining healthy hives. Beekeeping education could help improve overwintering survival among back yard beekeepers. To evaluate the role of education in successful beekeeping, in Summer 2018 and Summer 2019, backyard beekeepers across the state of Colorado were contacted to participate in a honey bee health survey that included a questionnaire and a hive inspection. Using hive management, beekeeper education, mite load, and experience as predictors of hive survival, this study found that hive survival may be positively related to hive management.

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## Chapter one

### Bee Diversity and Abundance Under A Grazed Cover Cropping Management System in Eastern Colorado and Southwestern Nebraska

#### **Introduction**

Agroecosystems account for 40% of the earth's surface and, in addition to provisioning major ecosystem services, are also major sources of ecosystem service consumption (Robertson and Swinton, 2005; Power, 2010; Schipanski et al., 2014). Humans manage agroecosystems intensively in order to produce food, fiber, pharmaceuticals, and biofuels. While management allows these systems to be productive, agroecosystems still depend on natural ecosystem processes for supporting services such as moisture retention and regeneration, pollination, soil fertility, nutrient cycling, and biodiversity (Robertson and Swinton 2005; Power 2010). Although a production-based agroecosystem, where maximizing yield and short-term profitability, may be efficient in its ability to produce feed, fiber, or forage, this often comes at the expense of other ecological services as well as demand for increased inputs such as fertilizer and irrigation (Robertson and Swinton, 2005; Bennett and Balvanera, 2007; Tscharntke et al., 2012). As such, enhancing the ability of an agroecosystem to provide ecosystem services could help offset production demands on the environment.

Definitions for ecosystem services vary but are generally services provided by an environment that are regulatory, provisionary, supportive or culturally valuable (Fisher et al., 2009; Braat and Groot, 2012; Schipanski et al., 2014; Porter and Francis, 2017; La Notte et al., 2017). As agriculture continues to be one of the dominant uses of land on the planet, creating holistic multifunctional agroecosystems that allow farmers to profit from both production as



well as from ecosystem services has become increasingly attractive (Tilman et al., 2011; Brown et al., 2012; Groot et al., 2012; Sayer and Cassman, 2013; Costanza et al., 2014).

Within the rangeland of the semi-arid short grass steppe ecosystem in eastern Colorado and southwestern Nebraska, wheat production represents the main dryland cropping option (Lauenroth et al., 1999; Lauenroth and Burke, 2008). Traditionally, wheat is grown in a summer fallow rotational system that produces a crop once a year and remains fallow during the alternative year, allowing for moisture regeneration (Lauenroth et al., 2000; Vick et al., 2016). While allowing for a fallow period within the field promotes moisture regeneration at little to no cost to the producer, fallow also depletes soil carbon and increases erosion (Vick et al., 2016). As a result, cover crop incorporation into agroecosystems as an alternative to fallow has been posited as an opportunity for increasing ecosystem services (Schipanski et al., 2014). Cover crop use can potentially enhance many ecosystem services including sequestering soil organic carbon, increasing nitrogen fixation, pest control, improving soil composition, reducing erosion, and increasing water capacity (Tonitto et al., 2005; Blanco-Canqui et al., 2013).

Pollination provided by bees is an important ecosystem service that facilitates reproduction for both wild and managed plants (Klein et al., 2007; Gallai et al., 2009; Potts et al., 2010; Bauer and Wing, 2016). Agricultural intensification and related habitat loss are two major stressors associated with the decline of bee health, diversity and abundance (Kremen et al., 2002; Nicolson and Wright, 2017; Arathi et al., 2019; Feltham et al., 2015). Depending on the mixture, cover crops could potentially benefit bee communities by providing habitat and forage. There are many studies that show the importance of including habitat for pollinators within agroecosystems and, if cover crops are able to provide this habitat, it may give further

incentive for farmers to adopt their use as a vibrant bee community near agricultural fields and could improve pollination-dependent cash crop productivity (Cane, 2011; Mandelik et al., 2012; Ellis and Barbercheck, 2015; Feltham et al., 2015; O'Brien and Arathi, 2018).

### **Cover crop multifunctionality, cattle grazing, and bees:**

Although cover crops may have the potential to enhance ecosystem services that provide long term benefits to farmers and the environment, farm viability with cover-cropping depends on the ability to generate competitive income. Thus, mixing of complementary activities including cattle grazing, could provide profitable means of generating income by spreading operating costs across multiple activities (Russelle et al., 2007; Brown et al., 2012). For rotational systems that incorporate cattle grazing, a cover crop rotation could present an additional opportunity in functionality through grazing prior to wheat planting. Currently, the dominant vegetation within the steppe is a mix of C<sub>3</sub> and C<sub>4</sub> grasses with C<sub>3</sub> dominating north of the Great Plains and two C<sub>4</sub> perennial grasses, *Bouteloua gracilis* (H. B. K.) Lag. ex Steud. (blue grama) and *Buchloë dactyloides* (Nutt.) Engelm. (buffalograss) dominating the south (Quinn et al., 1994; Tieszen, 1997). Approximately 70% of the short grass steppe remains in natural vegetation and is primarily used for cattle grazing (Lauenroth and Burke, 2008). Continuous grazing poses concerns to the ecosystem due to overexploitation and related problems. Overgrazing can negatively affect plant and insect community composition, contribute to soil erosion, and alter ecosystem functionality (Fleischner 1994; Dennis et al., 1998; Milchunas et al., 1998; Yoshihara et al., 2008).

A serious challenge that growers face when cultivating cover crops in arid and semi-arid dryland agriculture is reduction in soil moisture due to cover crop growth (Nielsen and Vigil

2005; Blackshaw et al., 2010). Grazing cover crops prior to wheat production could potentially recoup the cost of the cover crop mixture and the loss of soil moisture by gains made in cattle weight *via* grazing and allow grassland to regenerate elsewhere as cattle are moved around. While bee communities may benefit from cover crops, grazing has been reported to have both positive and negative effects on arthropod communities making it unclear how grazing cover crops may affect bees in these systems (Milchunas et al., 1998; Davis et al., 2014; Birkhofer et al., 2017). Evaluating the potential benefits that cover crops may have for bees within grazed dryland wheat agroecosystems of eastern Colorado and southwestern Nebraska will require establishing a baseline for bee diversity and abundance. Pollinator abundance in grazed semi-arid pastures has been shown to be dependent on the forage mixture grown in the field (Bhandari et al., 2018). Likewise, cover crop success in enhancing pollinator abundance has been shown to be mixture dependent as well (Ellis and Barbercheck, 2015). However, for the semi-arid regions of eastern Colorado there is little information on the bee diversity and hence the effects of cover cropping and grazing on this diversity. This study is the first attempt to quantify bee diversity and abundance in the eastern Colorado and southwestern Nebraska dryland region in an annual pre-wheat cover crop system, using a uniform cover crop mixture among three producer fields with grazing incorporated as a farm management practice.

## **Methods and Materials**

### **Study area:**

The locations of the farmer fields are proprietary and the names of landowners are redacted to maintain their privacy. Each field is identified by the first letter of the county where the field is

located: Weld County, Colorado (W), Kit Carson County, Colorado (K), and Perkins County, Nebraska (P). The geographic location and coordinates are presented in Figure 1.1 and Table 1.1.

#### **Field layout and trap locations:**

Prior to planting, seeds of the cover crop mixture were purchased in partnership with Green Cover Seed (Green Cover Seed LLC, Bladen, Nebraska). The cover crops included oats, barley, triticale, peas, flax, safflower, black oil sunflower seeds, rapeseed, purple top turnip, and millet (Table 1.2). Cover crops were planted in early in late March through early April and grazed for approximately one month between June and July before cattle were removed from the field and the cover crop terminated (ploughed) by the grower for the subsequent planting of wheat. A total of 27 SpringStar blue vane traps (Springstar, Inc., Woodinville, Washington) were placed in the pre-wheat cover crop fields (Figures 1.2-1.5). Although trapping methods vary in efficacy for capturing bees (Joshi et al., 2015; O'Brien and Arathi, 2018; O'Brien and Arathi, 2019), blue vane traps have been documented to be more useful in broader biodiversity studies, effectively trapping a greater diversity of bees than other passive trapping methods (Kimoto et al., 2012; Joshi et al., 2015; Gibbs et al., 2017; Hall, 2018). Targeted trapping was not possible in our study as the cover crops were grazed prior to flowering.

#### **Treatments:**

As shown in the schematic diagram in Figure 1.2, each field included four replicate plots within which there were three management regimes, with the exception of P field that did not establish all the three regimes due to weather interruptions (Table 1.2).

- i. Grazed: Cattle were allowed to graze freely across replicates.

- ii. Ungrazed: ~2,200m<sup>2</sup> fenced off enclosure where cover crop was inaccessible to cattle.
- iii. Fallow: Within the ungrazed management regime, an approximate 21m<sup>2</sup> area was sprayed with herbicide to kill the cover crop.

**Trap layout:**

Each field had a total of nine traps placed in clusters of set in a triangular pattern (Figure 1.6).

Each cluster was placed in one of the three management regimes such that there was one cluster of traps in the grazed, one in the ungrazed, and one in the fallow. The traps were each assigned a number 1-9, the location marked with a flag, and GPS coordinates recorded to help locate them as the cover crops grew and cattle foraged through the grazed area. Each trap was activated once a month for seven consecutive days. At the end of the seventh day, the traps were closed. The vanes of the trap were removed and laid flat across the opening of the bottom half of the traps and then wrapped in a large plastic trash bag to prevent entry into traps outside of the designated collection period.

**Collection schedule:**

Bees were collected in a pre-wheat cover crop rotation. Field sites chosen were based on individual grower/stakeholder participation and grazing, its frequency and duration varied by producers and the weather conditions during that year. Details of planting and grazing schedules are presented in Table 1.1. Bees were sampled in each field and sampling frequency varied based on farm management practices. In two of the fields, collections as described above were completed three times in the season with the exception of K field because it was terminated early. In the P field, where the farm management regimes were not instituted, the collection was different. See Table 1.3 for detailed collection schedules across each field site.

**Bee specimen processing and identification:**

All bees were removed from blue vane traps and placed into plastic bags labeled with the corresponding trap ID, date, and site information. Each plastic bag was placed into an ice cooler and transferred to the laboratory where specimens belonging to Apoidea were separated and washed in acetone to remove any debris. The specimens were then dried and pinned.

Specimens were labeled appropriately indicating the necessary collection information, treatment and trap numbers. All Apoidea specimens were identified to genus level and species identification was completed when identification resources were available (Table 1.4). While bycatch was recorded, all non-bee specimens were only identified to level of order. Bee specimen identifications were verified by Dr. Boris Kondratieff – Director of the C.P. Gillette Museum of Arthropod Diversity (Colorado State University), Virginia Scott – Collections Manager of Entomology (University of Colorado, Boulder), and Dr. Adrian Carper - Postdoctoral Research Associate Department of Ecology and Evolutionary Biology (University of Colorado, Boulder).

**Diversity and abundance measures:**

Shannon-Weiner index ( $H' = -\sum_i^R p_i \ln p_i$ ) and Simpson's index ( $D = 1/\sum_i^R p_i^2$ ) were the diversity measures used for different months within a field and for values between field locations. In the Shannon-Weiner index,  $p$  equals the proportion ( $n/N$ ) of collected individuals in one genus ( $n$ ) divided by total individuals ( $N$ ) in the sample,  $\ln$  is the natural log,  $\sum$  is the sum of all  $p$  values from 1 to  $R$  across the  $i^{\text{th}}$  (respective) genera in the sample, and  $R$  equals the total number of genera in the sample. In Simpson's index,  $p$  is the proportion ( $n/N$ ) of collected individuals belonging to one genus ( $n$ ), divided by the total number of individuals ( $N$ ) found in

the sample,  $\sum$  is the sum of all  $p$  values 1 to R across the  $i^{th}$  (respective) genera in the sample, and R is the total number of genera in the sample. The Shannon-Weiner index measures both evenness and richness, assuming that all genera are represented in a sample while the Simpson index accounts for the greater abundance of common genera assuming that the rare ones with only a few representative individual bees will not affect the diversity values. Larger values indicate greater diversity (Krebs, 1989).

Sorenson's coefficient is used to calculate community similarity ( $CC = \frac{2C}{(S1+S2)}$ ), where C represents the number of genera that are the same between two communities, S1 is the number of total genera in one field or one collection month, and S2 is the total number of genera from a second field or the second collection month. The coefficient was calculated to determine the extent of overlap of bee genera for each month within each field as well as to determine overlap between the three fields. Coefficients with values closer to 1 refer to fields that have greater community similarity while fields with coefficients values closer to 0 refer to fields that have lower community similarity.

## Results

A total of 5,331 individual bees belonging to 36 genera were collected from the three fields in Colorado and Nebraska (Table 1.4). Of these, 2,700 individuals, nearly 51% of the total number of bees collected during the study was comprised of the species rich genus *Lasioglossum* (Halictidae) but the abundance of this genus varied across the three fields. Whereas, *Lasioglossum* was the most abundant genus in both W (~42%) and K (~72%) fields, it was only third in abundance in the largely ungrazed P field (~13%) (Figure 1.7). The sunflower

bee in the genus *Melissodes* was the most abundant in P field (~38%), followed by *Svastra* (~17%), a genus that was absent from both W and K fields (Figure 1.8). P field had the highest diversity of collected bees with 29 total genera as well as the highest number of genera unique to that field but had the lowest overall abundance (Figures 1.9 and 1.10). K field had the second highest diversity with 26 total genera, four that were unique to K field, and had the greatest abundance of bees, with the genus, *Lasioglossum* being the most abundant (Figures 1.11 and 1.12). Twenty-two genera were collected in W field, two unique, and total abundance was primarily dominated by *Lasioglossum* (Figures 1.13 and 1.14).

#### **Diversity and Abundance measures:**

The Shannon diversity index ( $H'$ ) values ranged from 2.17 for P field to 1.98 for W field and 1.22 for K field. In regard to the Simpson index ( $D$ ), the results followed a similar pattern with the highest value being P field (4.96), the second highest being W field (4.40), and the lowest value being for K field (1.90) (Figures 1.15). Indices varied between fields but were more similar within fields except when using Simpson's index for the grazed regime in W field,  $D=3.50$ , compared to the fallow,  $D= 4.62$  and ungrazed,  $D= 4.72$  (Figures 1.16). Community similarity calculated by Sorenson's coefficient indicated that P and K fields were most similar,  $CC=0.764$  with 21 overlapping genera, followed by W and K fields,  $CC=0.750$  with 18 overlapping genera, and W and P field were least similar,  $CC=0.745$  with 19 genera (Table 1.5) (Figure 1.17).

#### **Seasonal changes to diversity:**

Seasonal shift was determined by calculating the monthly change in diversity for each field. Sorenson's coefficient was used to calculate community similarity between months at each field location (Table 1.6). During the month of May, 14 genera were collected in W field while



19 were collected from K field and 20 were collected from P field. During the month of May, W and K field had 11 overlapping genera, W and P had 12 overlapping genera, and K and P had 15 overlapping genera. W and K field shared  $H' = 1.03$  and D were more similar for W and P during this month and were least similar for P and K. P field had the highest value for both  $H' = 2.03$  and  $D = 4.74$  while K field had the lowest value of  $H' = 1.03$  and  $D = 1.73$ . During the collection period for June the genera collected from W field increased to 19, P field remained at 20 genera, and K field decreased to 18. W and K shared 14 genera in June as did W and P while K and P fields shared 12 overlapping genera. W and K fields had 10 of the same genera that had been collected in May as did W and P field, however K and P field had only nine of the same genera. For values that included population evenness, again W and P field were most similar and P and K were least similar. P field had the greatest D value of 1.93 and  $H'$  was greatest in W field with a value of 4.08. Twenty genera were collected at P field, but  $H'$  declined to 1.93 and D to 3.37. During the final collection period 18 genera were collected in July W field and P field the number of genera declined from 20 genera to 12 genera.

The Sorenson's coefficient was calculated for each field to indicate differences of community for each month (Figures 1.17). Because the cover crop was terminated early in K field, there was no data for July and only a seasonal comparison could be made for overlapping genera in May and June (Figure 1.17). In W field, May and June had 12 overlapping genera ( $C = 0.727$ ), May and July had 11 overlapping genera ( $CC = 0.689$ ), and June and July had 16 overlapping genera ( $CC = 0.865$ ). In P field May and June shared 13 overlapping genera ( $CC = 0.650$ ), May and July had seven overlapping genera ( $CC = 0.438$ ), and June and July had eight

overlapping genera (CC=0.500). K field only had one coefficient for May and June, CC=0.595 with 11 overlapping genera as seen in Figure 1.17.

## **Discussion**

Bee diversity was higher in fields where no grazing allowed cover crops to flower. Whereas the proximity of trap clusters to one another and trap attractiveness may affect bee diversity and abundance within a field (Gibbs et al., 2017), thus making it difficult to determine the effect of regime, distinct differences in diversity of bee genera and several unique taxa at each study site, suggests that cover crop grazing may impact bee abundance and diversity. These differences can be separated into three probable factors: 1) grazing and its effect on the availability of floral resources and nesting habitat for bees; 2) cover crop mixture and its impact on bee foraging when cover crops are allowed to flower; 3) and finally seasonal and spatial variations in bee emergence and activity.

### **Grazing**

In all of the study fields, the same cover crop mix was planted but germination and plant establishment depended on field conditions. Due to lack of moisture needed to produce adequate biomass for grazing cattle, Perkins County (Nebraska) field was not grazed resulting in early flowering (*personal observation*) and the observed high values of Shannon-Weiner and Simpson's indices support the likelihood that increased floral availability can increase bee diversity.

However, Perkins county field also exhibited lowest abundance which could be explained by the drought-like conditions and inability of the environment to sustain increased

bee populations following inadequate nutritional resources (Phillips et al., 2018). Similarly, Weld county (Colorado) field had ungrazed areas that were allowed to flower which could also help explain the higher bee diversity of Weld county field compared to Kit Carson County (Colorado) field. Conversely, the Kit Carson County field had the largest abundance of bees but the lowest diversity despite that in this field, the cover crop never flowered and the cover crop was terminated prior to wheat planting. While it is not clear why this field had such high abundance of bees, it is possible that nesting conditions in this field, which did not experience drought conditions, may have been better depending on the taxa (Michener, 1964; Vulliamy et al., 2006; Kimoto et al., 2012).

The most abundant bees in Kit Carson County field were *Lasioglossum semicaeruleus* (Cockerell). There is evidence that *Lasioglossum* and other halictids prefer bare ground and compacted soil, that grazed fields tend to offer (Michener, 1964; Vulliamy et al., 2006; Kimoto et al., 2012). Conversely, some bees do not prefer these conditions including some members of the family Megachilidae (Michener, 1964; 2006; Kimoto et al., 2012). These bees do not dig their own burrows in the soil substrate but rather utilize materials at the soil surface, such as plant matter and stem cavities that may be disturbed by grazing cattle (Michener, 1964; Vulliamy et al., 2006; Kimoto et al., 2012). Three individuals of the genus *Megachile* were collected from Kit Carson County field, the lowest abundance of the three fields for this genus. Similar studies have also found that bumblebees are sensitive to grazing pressure and the lack of bumble bee abundance in the grazed fields in our study may be a result of females altering their behavior to exclude grazed areas (Kimoto et al., 2012). While grazing may have had an impact on soil condition and by extension ground nesting, this was not the focus of this study

and nesting conditions were not sampled therefore soil conditions resulting from grazing and the impact on bee abundance is strictly speculative.

#### **Cover crops mix:**

Although the cover crop mix was consistent across fields, Weld County and Perkins County fields allowed cover crops to flower, which may help explain the higher diversity index values for these two fields as opposed to Kit Carson County field. Bees receive their nutritional requirements from floral pollen and nectar and the growers included five flowering annuals in their mixes: flax, safflower, sunflower, pea, and rapeseed. While there was no determination of whether or not the bees collected from the traps had visited the flowers, the flowers may have been an attractant.

#### **Seasonal and spatial variation in emergence and behavior of bees:**

Soil and ground cover preferences for nesting as well as plant specialization may help elucidate the presence of bee genera such as *Lasioglossum* and *Melissodes*, but the presence of other bees may be explained by seasonal emergence while others may be the result of the geographical distribution of the genus (Michener, 1964; Hurd et al., 1980; Parker et al., 1981; Vulliamy et al., 2006; Kimoto et al., 2012). One such example is the presence of the chimney bee *Melitoma grisella* (Cockerell and Porter) in Perkins County field and its absence from the other fields. While it was lower in abundance than some of the other genera collected in Perkins County field, its occurrence may be related to its known geographical distribution which includes the Nebraska and Kansas border where Perkins County field is located (Linsley et al., 1980). While *Melitoma grisella* is known from Colorado (Scott et al., 2011), this species is considered uncommon in the state but becomes more common eastward near the Kansas and

Nebraska borders (Wilson and Carril, 2016). Additionally, of the Eucerini bees, *Eucera* is considered an early-mid season bee whereas *Melissodes* is considered a mid-late season bee (Parker et al., 1981; Wilson and Carril, 2016). The K field had the lowest abundance of *Melissodes* as compared to the other fields. Trapping continued into late July at Perkins County field and had the highest abundance in number of *Melissodes* collected which in part may have been due to preferential foraging for sunflowers that are available later in the season but also may have been influenced by a seasonal shift in population dynamics that could not have been detected in W and K fields (Robertson, 1926; Hurd et al., 1980; Parker et al., 1981).

### **Managerial Considerations**

The use of cover crops in grazed agroecosystems as a potential resource for native bees should be mutually beneficial to the grower and to ecosystem services. A greater diversity of flowering plants left ungrazed may benefit diverse community of bees but there is evidence that bees such as the Halictidae prefer grazed field conditions (Kimoto et al., 2012). Furthermore, grazing is necessary to help support the economic needs of the grower as the multi-functionality of a farm helps ensure its viability (Brown et al., 2012). While completely grazing a field may benefit a few genera, halictids in particular (Michener, 1964; Vulliamy et al., 2006; Kimoto et al., 2012), having higher cattle density that out grazes available forage can have negative effects on bee diversity. Intensive grazing results in a lack of floral diversity and tends to favor generalist bee species, decreasing native bee diversity (Danforth et al., 2019). As a consideration for growers, although it may not be practical to completely remove a field from a grazing rotation, planting a diverse flowering cover crop in areas that remain ungrazed could help support greater bee diversity.

## Tables

<b>Table 1.1:</b> Location, County, field area, planting date, and grazing schedule.							
*Field	Location	County	Field Area (ha)	Planting Date	Cattle on field	Cattle off field	Days Grazed
W	Raymer, CO	Weld	17.2	March 23, 2017	June 22, 2017	July 27, 2017	35
K	Seibert, CO	Kit Carson	40.5	March 14, 2017	June 15, 2017	July 6, 2017	21
P	Venango, NE	Perkins	36.7	April 4, 2017	--	--	--
<p>* W: Weld County, Colorado field, K: Kit Carson County, Colorado field, P: Perkins County, Nebraska field.</p> <p>-- Perkins County field was not grazed due to weather conditions during the study.</p>							

<b>Table 1.2:</b> Cover crop species mix.			
Crop	Species name	Bee-friendly	Flowering year (bee-friendly plants only)
Oats	<i>Avena sativa</i>	No	--
Barley	<i>Hordeum vulgare</i>	No	--
Triticale	<i>× Triticosecale</i>	No	--
Millet	<i>Panicum miliaceum</i>	No	--
Peas	<i>Pisum sativum</i>	Yes	First
Flax	<i>Linum usitatissimum</i>	Yes	First
Safflower	<i>Carthamus tinctorius</i>	Yes	First
Sunflower	<i>Helianthus annuus</i>	Yes	First
Rapeseed	<i>Brassica napus</i>	Yes	First
Purple Top Turnip	<i>Brassica rapa</i>	Yes	Second
--Flowering year not included for grasses.			

**Table 1.3:** Trapping information and sampling schedule for treatments, Weld County (W), Colorado; Kit Carson County (K), Colorado; Perkins County (P), Nebraska.

*Field	Treatment	Trap numbers	Field Regime	Coordinates	Collection Round	Sample Date Range
W	Fallow	4-6	2	40.503, -103.901	1	May 24 2017- June 01 2017
	Grazed	1-3	1	40.504, -103.898	2	June 21 2017-June 28 2017
	Ungrazed	7-9	4	40.503, -103.903	3	July 05 2017– July 12 2017
K	Fallow	1-3	1	39.210, -102.876	1	May 24 2017- June 01 2017
	Grazed	7-9	4	39.210, -102.884	2	June 21 2017-June 28 2017
	Ungrazed	4-6	3	39.210, -102.883	3 (terminated)	--
P	Fallow	1-3	3	40.799, -101.943	1	May 24 2017- June 01 2017
	**1 (ungrazed)	4-6	4	40.799, -101.944	2	June 21 2017-June 28 2017
	2 (ungrazed)	7-9	1	40.799, -101.936	3	July 21 2017 – July 27 2017
<p>* W: Weld County, Colorado field, K: Kit Carson County, Colorado field, P: Perkins County, Nebraska field.</p> <p>** Weather conditions prevented grazing within P field thus treatments were renamed 1 and 2 and treated as a single ungrazed treatment.</p> <p>--No data available</p>						



<b>Table 1.4:</b> Total abundance and bee diversity, Weld County (W), Colorado; Kit Carson County (K), Colorado; Perkins County (P), Nebraska.			
Genus	W field (CO)	K field (CO)	P field (NE)
<i>Agapostemon</i>	305	59	10
<i>Anthidium</i>	7	1	0
<i>Anthophora</i> <i>affabilis</i> <i>montana</i> <i>occidentalis</i> <i>walshii</i>	40	42	9
<i>Apis</i> *** <i>mellifera</i>	0	0	2
<i>Augochlorella</i>	0	1	3
<i>Augochloropsis</i>	2	5	3
<i>Bombus</i> <i>huntii</i> <i>pennsylvanicus</i>	60	58	21
<i>Calliopsis</i>	20	1	2
<i>Ceratina</i> **	0	1	0
<i>Colletes</i>	0	1	2
<i>Diadasia</i> <i>enavata</i>	27	16	20
<i>Dianthidium</i> ***	0	0	1
<i>Epeolus</i> ***	0	0	5
<i>Eucera</i> <i>hamata</i> <i>lepida</i> <i>pallidihirta</i> <i>speciosa</i>	260	223	68
<i>Habropoda</i> <i>morrisoni</i>	16	0	6
<i>Halictus</i> <i>parallelus</i>	80	60	16
<i>Hoplitis</i>	17	11	17
<i>Hylaeus</i> ***	0	0	1
<i>Lasioglossum</i> <i>semicaeruleus</i>	812	1766	122
<i>Lithurgopsis</i> * <i>apicalis</i>	10	0	0
<i>Megachile</i>	15	3	19
<i>Melissodes</i>			

<i>agilis</i>	140	82	354
<i>Melitoma</i> *** <i>grisella</i>	0	0	2
<i>Neolarra</i> **	0	1	0
<i>Nomada</i>	4	6	11
<i>Nomia</i> * <i>universitatis</i>	3	0	0
<i>Osmia</i>	69	40	14
<i>Panurginus</i> **	0	1	0
<i>Perdita</i>	33	6	15
<i>Protandrena</i> <i>abdominalis</i> <i>bancrofti</i>	4	0	4
<i>Pseudopanurgus</i>	0	1	2
<i>Sphecodes</i>	19	75	14
<i>Stelis</i>	0	1	1
<i>Svastra</i> *** <i>obliqua</i>	0	0	153
<i>Triepeolus</i>	1	2	26
<i>Xenoglossa</i> **	0	1	0
<b>Total</b>	<b>1944</b>	<b>2464</b>	<b>923</b>
<b>Unique</b>	<b>2</b>	<b>4</b>	<b>6</b>
* Genera unique to W field ** Genera unique to K field ***Genera unique to P field			

**Table 1.5:** Sorenson coefficient (CC) comparing overlapping genera between fields to determine community similarity.

Total genera for each field	Compared fields	# of overlapping genera of compared fields	Sorenson coefficient (CC)
W =22	W-P	19	0.745
K=26	W-K	18	0.75
P=29	P-K	21	0.764

**Table 1.6:** Seasonal changes in Shannon-Weiner (H') and Simpson's diversity indices (D) and Sorenson's coefficient (CC) for community similarity between months.

*Field	Month	# of genera	# of overlapping genera	Shannon-Weiner (H')	Simpson's (D)	Sorenson's (CC)
W	May	14	May-June=12	1.53	2.97	May-June=0.727
W	June	19	June-July=16	1.85	4.08	May-July=0.689
W	July	18	May-July 11	2.04	4.79	June-July=0.865
P	May	20	May-June=13	2.03	4.74	May-June=0.650
P	June	20	June-July=8	1.93	3.37	May-July=0.438
P	July	12	May-July=7	1.14	2.40	June-July=0.500
K	May	19	May-June=11	1.03	1.73	May-June=0.595
K	June	18	--	1.49	2.77	--

\* W: Weld County, Colorado field, K: Kit Carson County, Colorado field, P: Perkins County, Nebraska field.

H': Shannon-Weiner diversity index value-higher values indicate greater diversity

D: Simpson index diversity index value-higher values indicate greater diversity with greater weight to common genera.

CC: Sorenson coefficient- values closer to 1 represent greater community similarity.

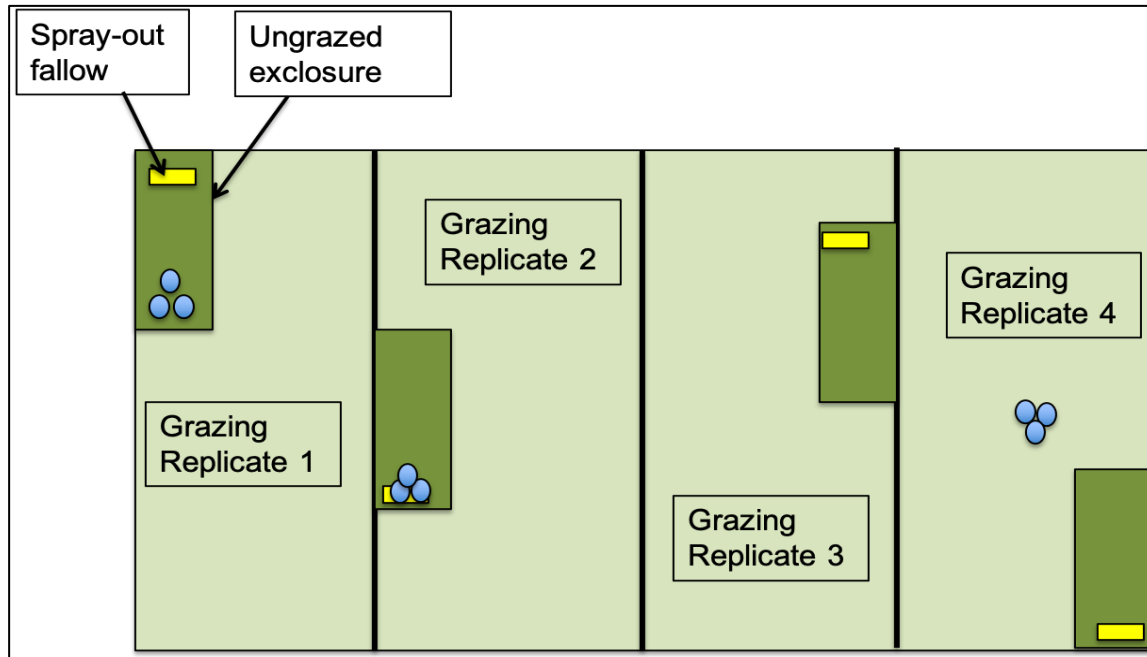
-- refers to data that is not applicable as a result of change in field management.

## Figures

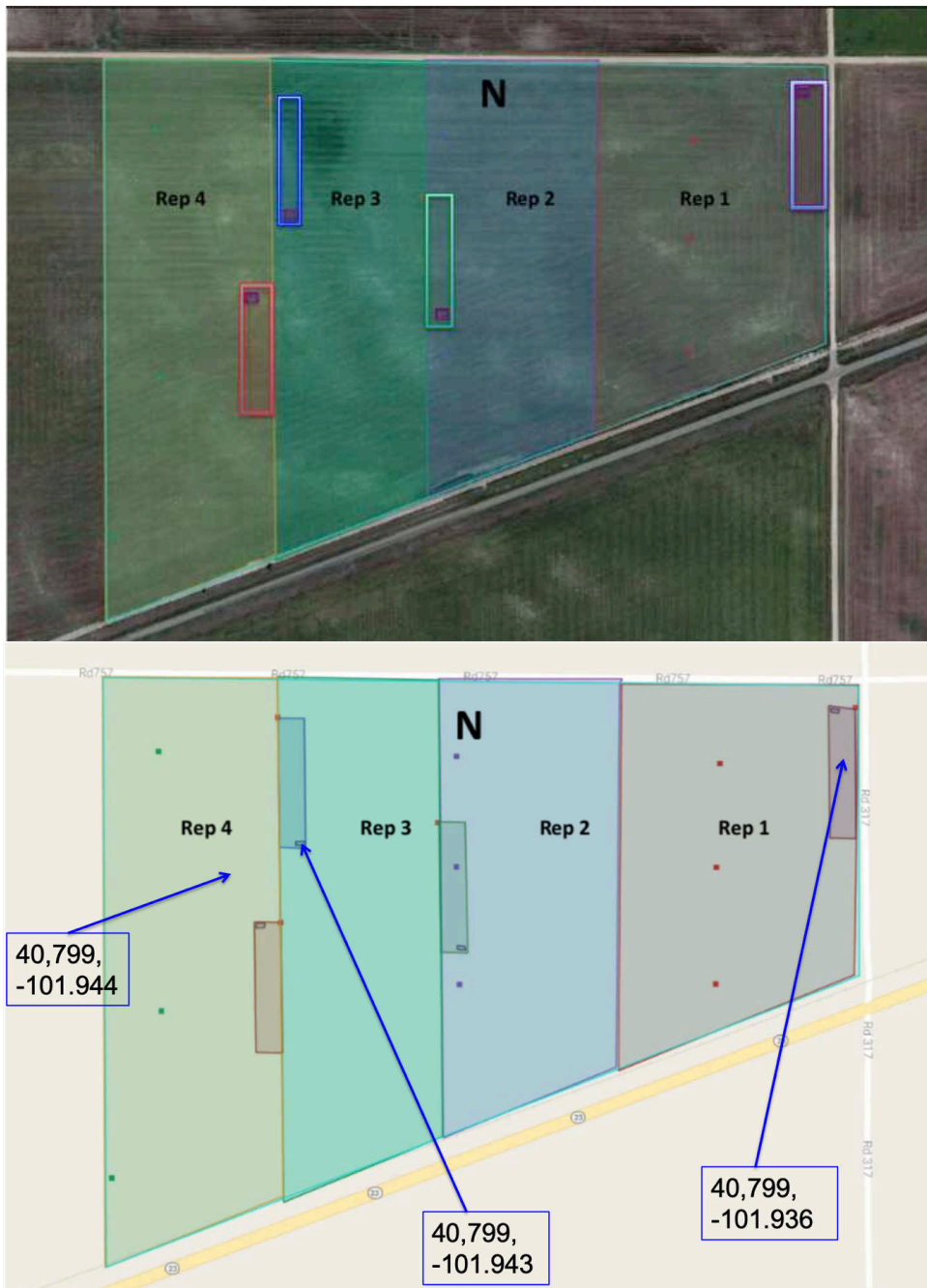
**Figure 1.1.** Location of the three field sites in Colorado and Nebraska.



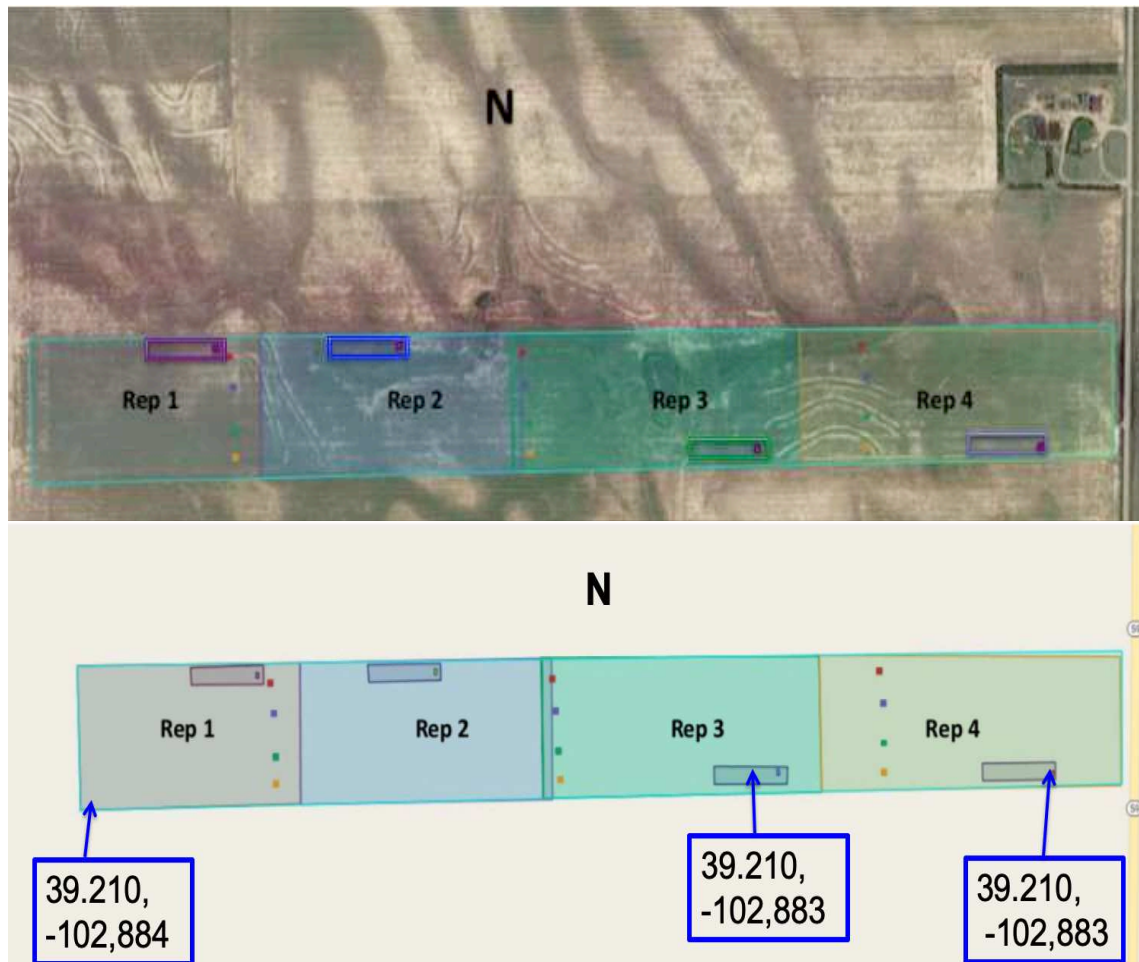
**Figure 1.2.** Schematic diagram of the field layout and trap locations. The herbicide sprayed fallow (yellow rectangle) and ungrazed enclosure (green rectangle) are located within the grazed treatment (gray rectangle) as indicated. Blue vane traps are represented by blue circles within the respective treatment locations in the field.



**Figure 1.3.** Perkins County, Nebraska satellite map and field layout with indicated trap coordinates.

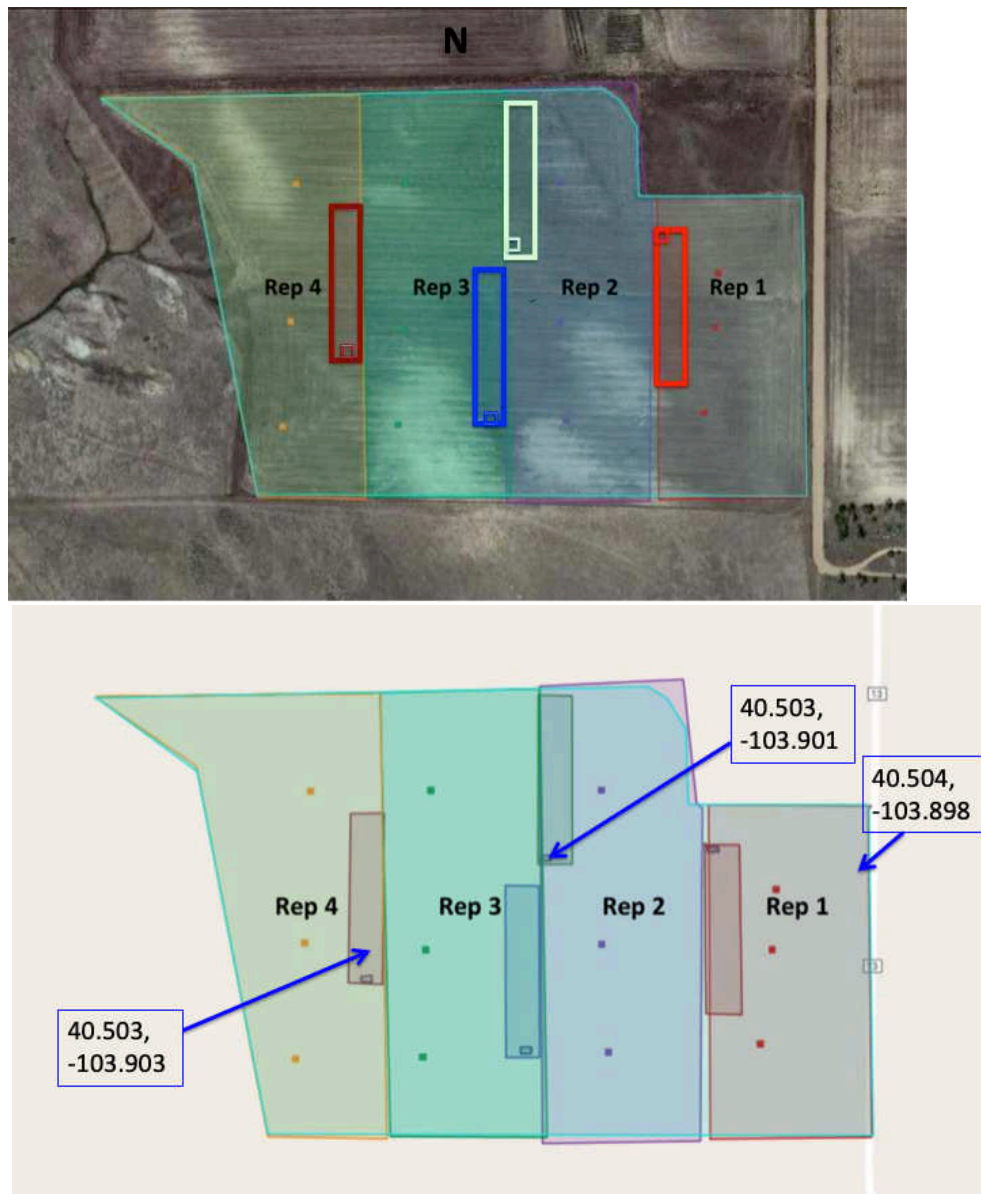


**Figure 1.4** Kit Carson County, Colorado field satellite map and field layout with indicated trap coordinates.





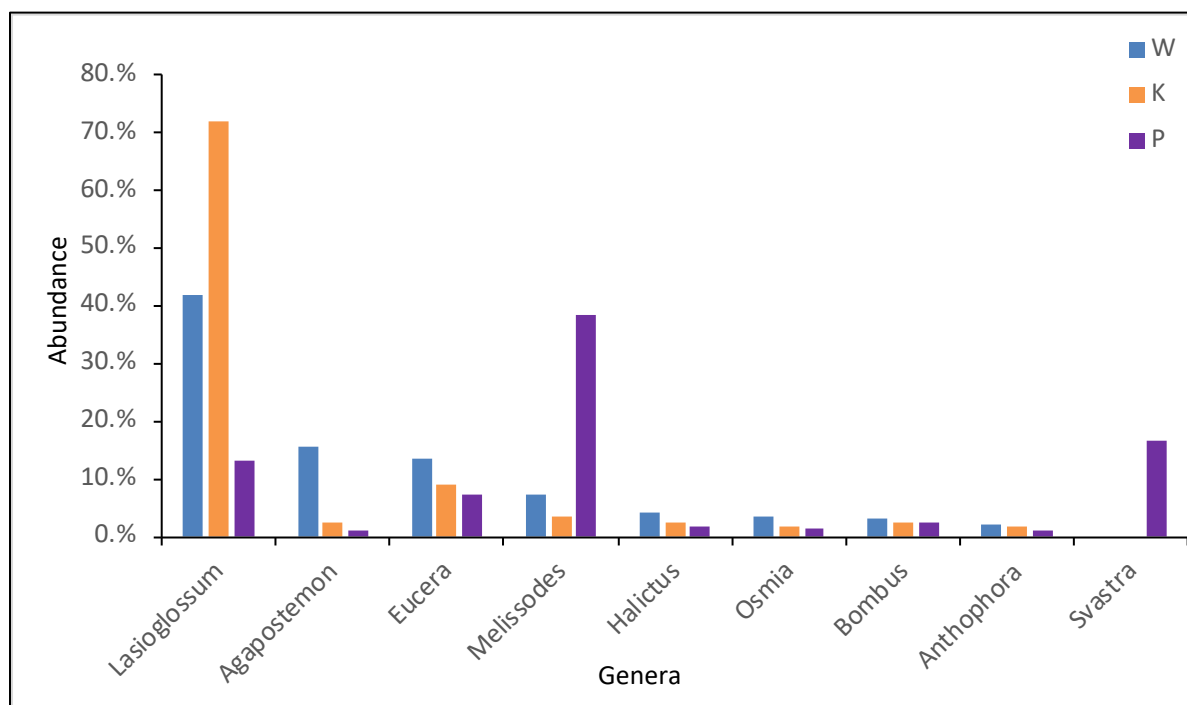
**Figure 1.5.** Weld County, Colorado field satellite map and field layout with indicated trap coordinates.



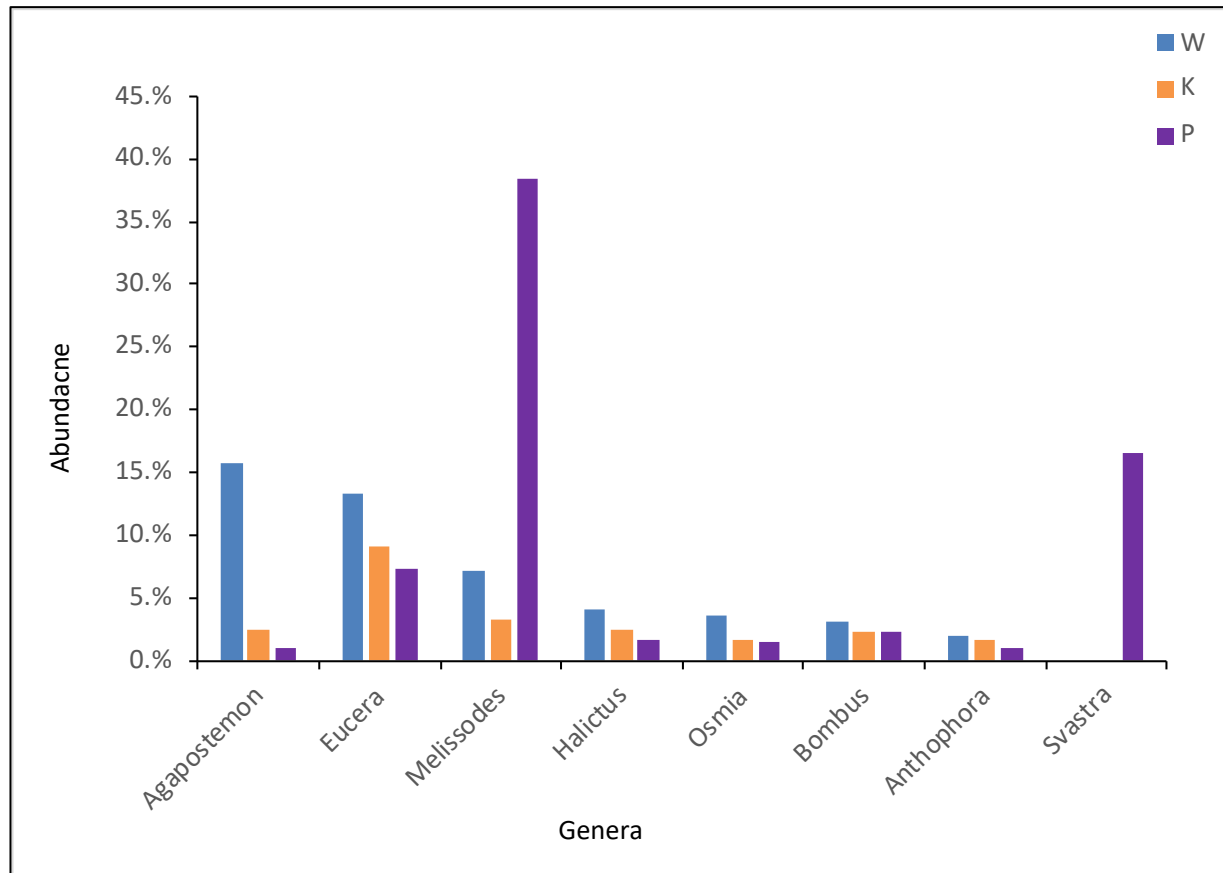
**Figure 1.6.** Open blue vane trap cluster. Traps were flagged to help locate traps as crops grew.



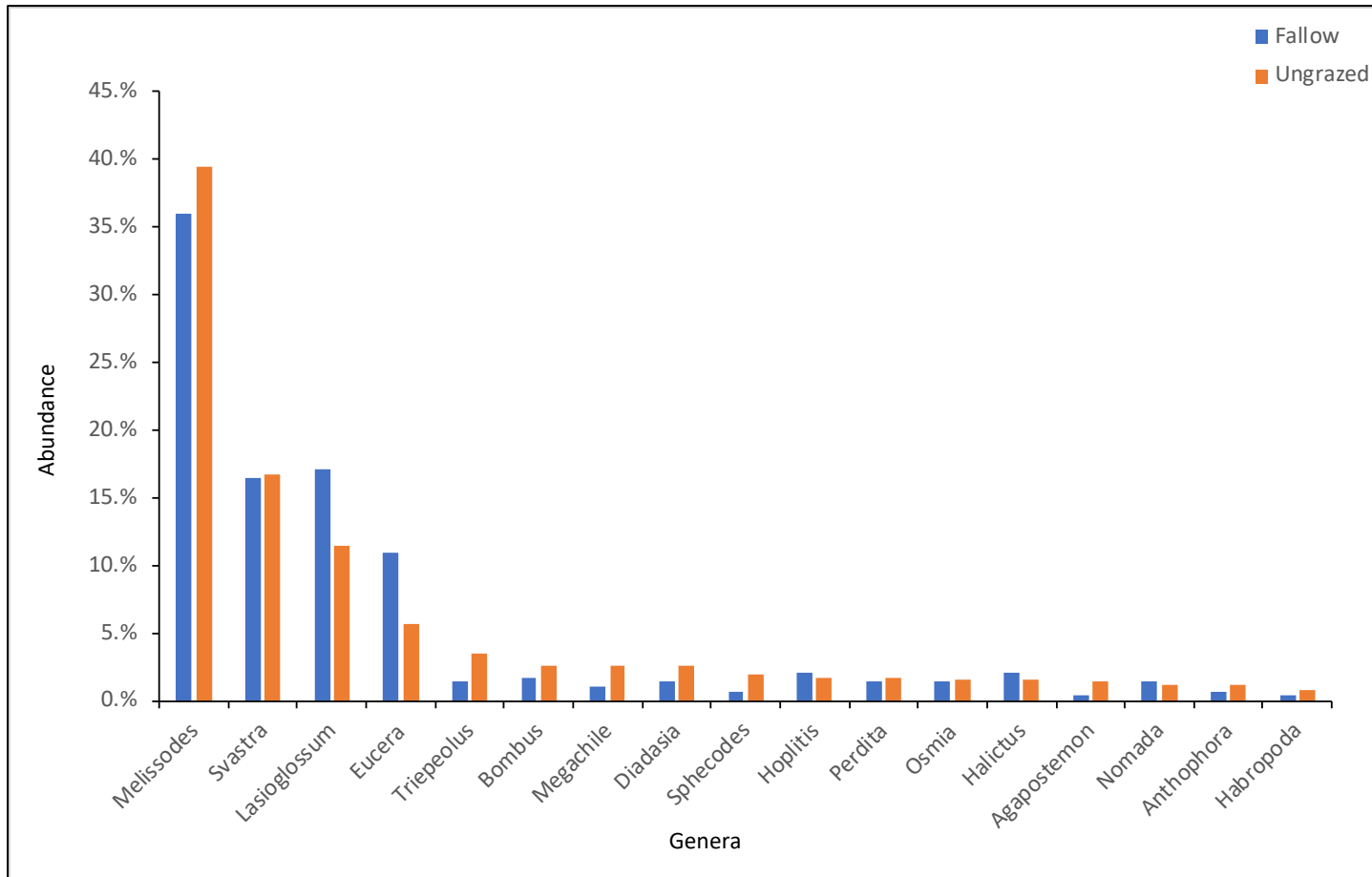
**Figure 1.7.** Bee diversity with abundance of five or more bees for Weld County, Colorado field (W), Kit Carson County, Colorado field (K), and Perkins County, Nebraska field (P).



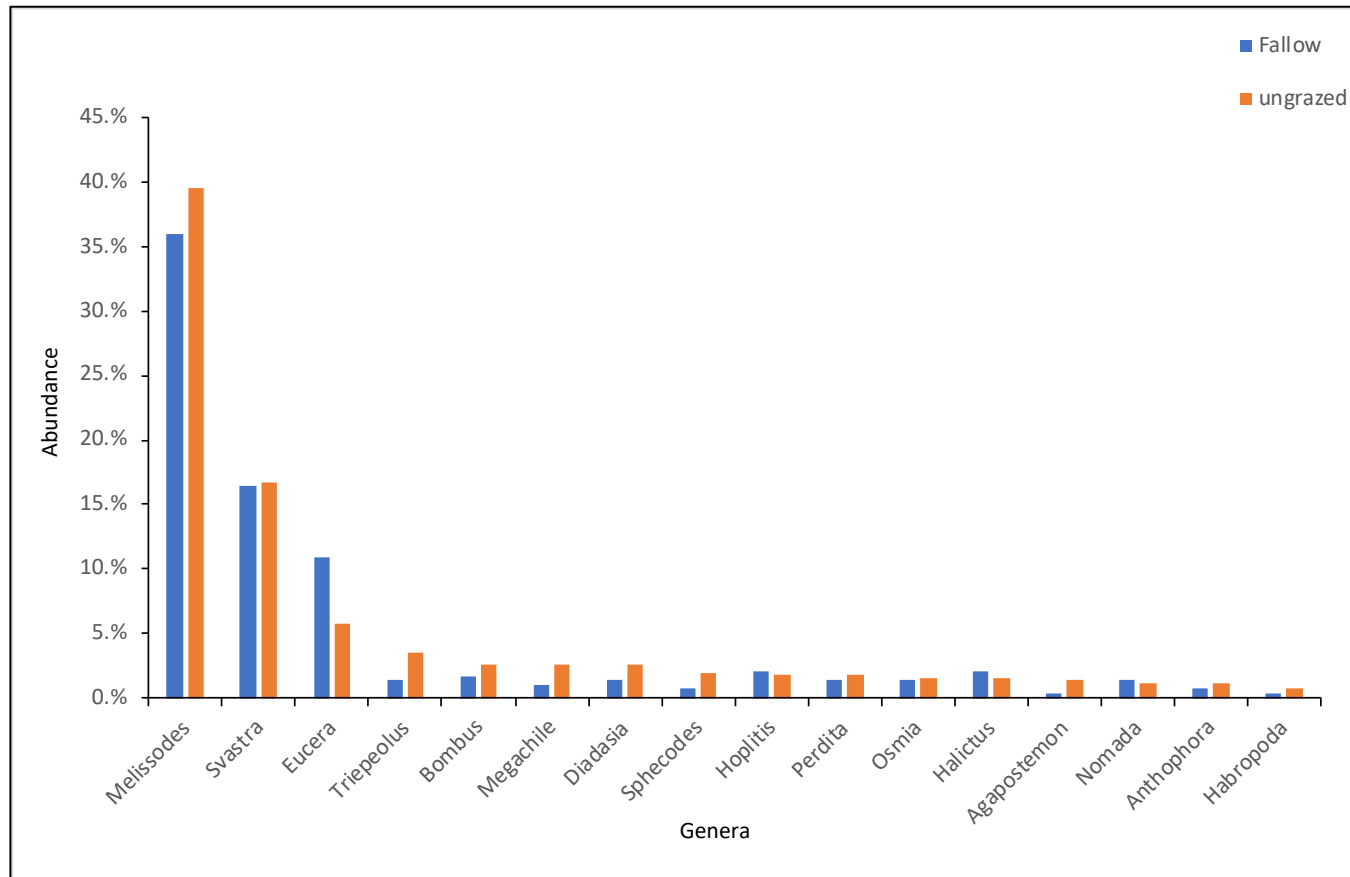
**Figure 1.8.** Bee diversity with abundance of five or more bees for Weld County, Colorado field (W), Kit Carson County, Colorado field (K), and Perkins County, Nebraska field (P) when *Lasioglossum* is excluded.



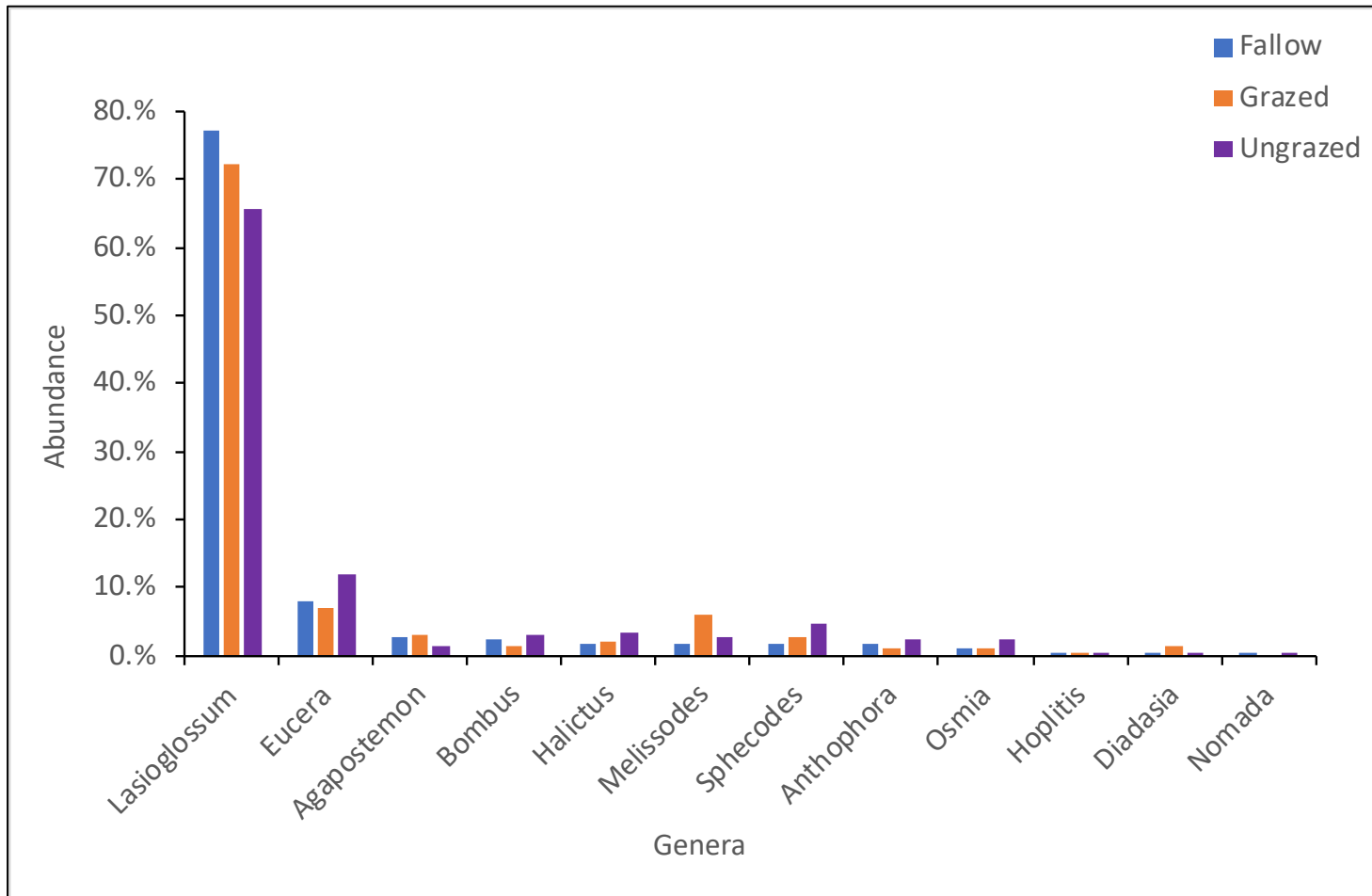
**Figure 1.9.** Perkins County, Nebraska diversity for bee genera with five or more individuals.



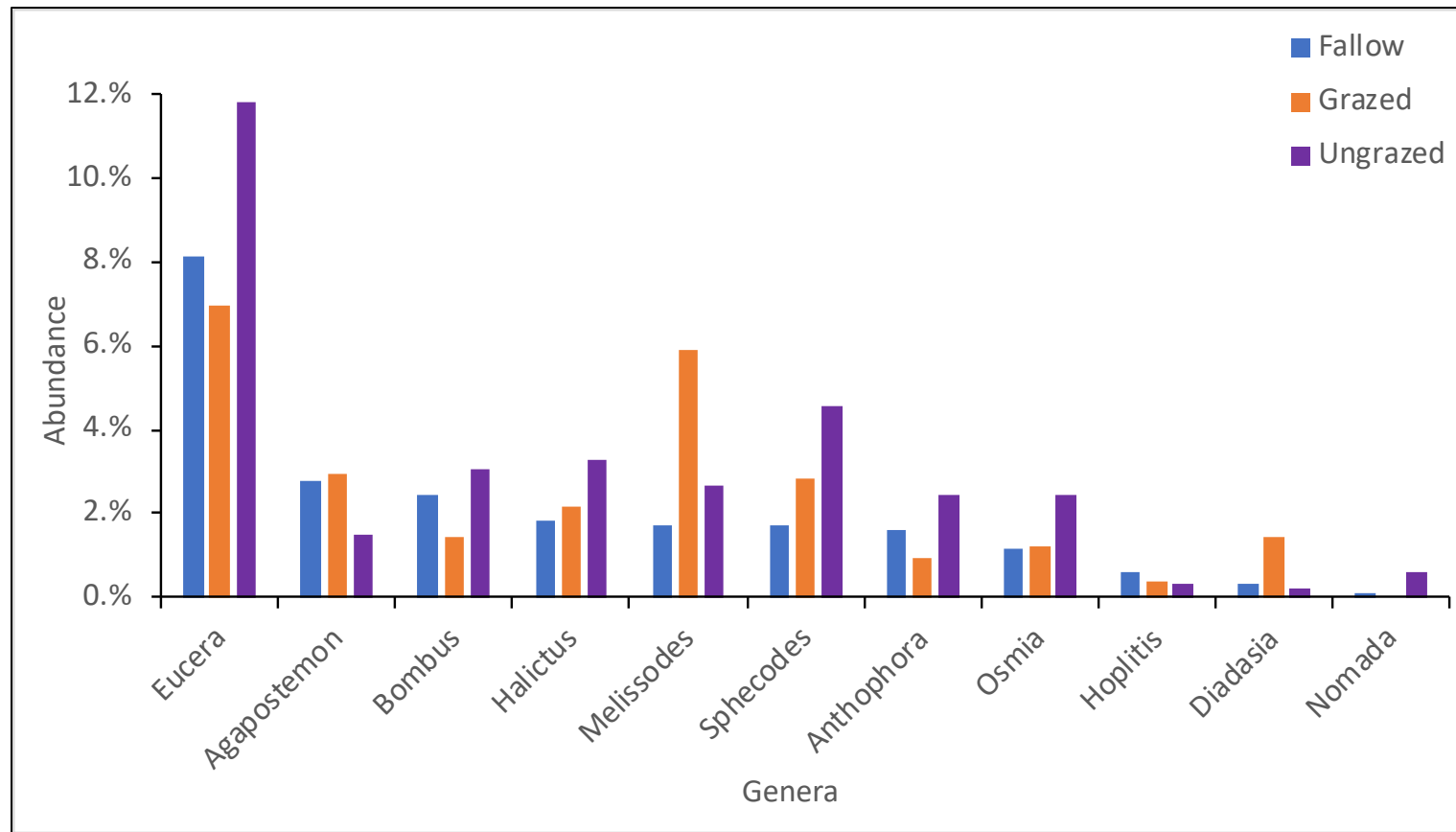
**Figure 1.10.** Perkins County, Nebraska diversity for bee genera with five or more individuals when *Lasioglossum* excluded.



**Figure 1.11.** Kit Carson County, Colorado diversity for bee genera with five or more individuals.

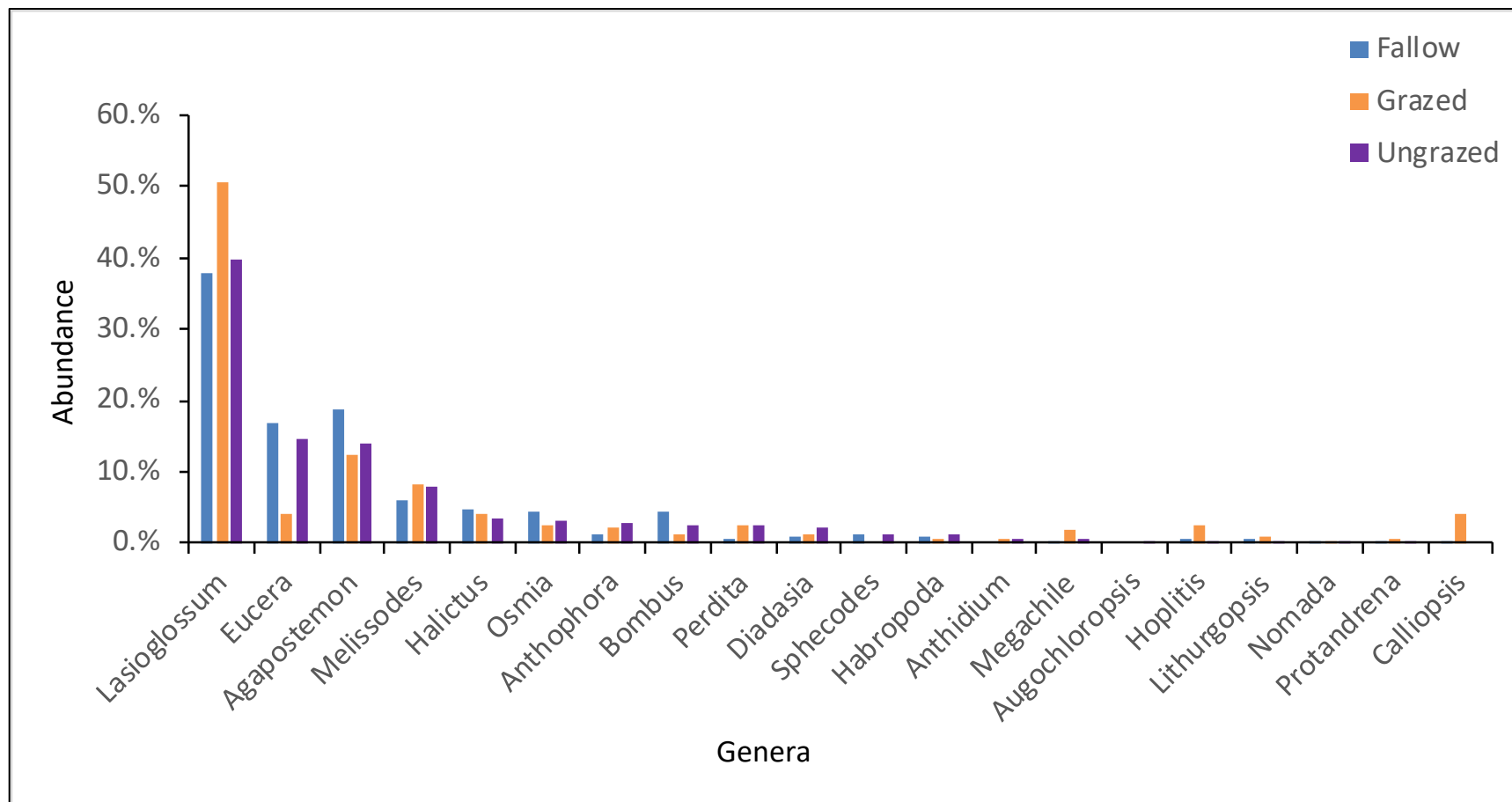


**Figure 1.12.** Kit Carson County, Colorado diversity for bee genera with five or more individuals when *Lasioglossum* excluded.

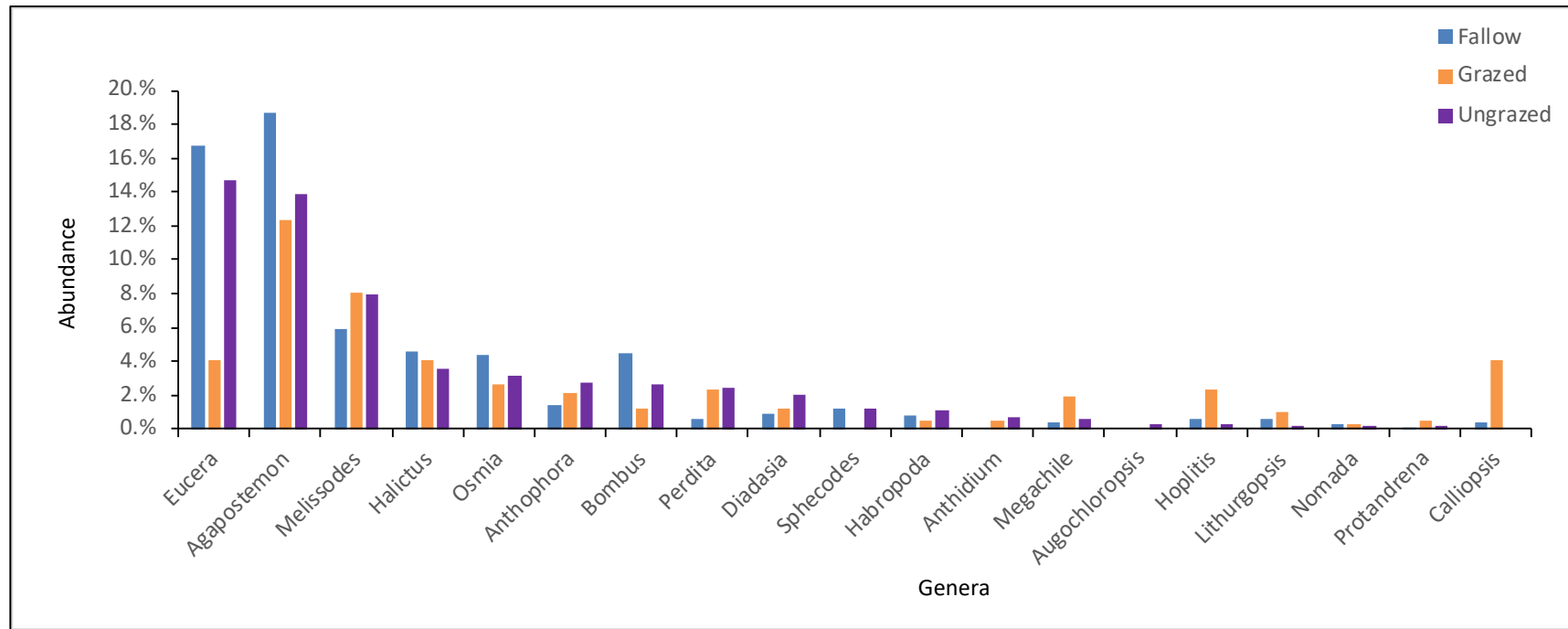




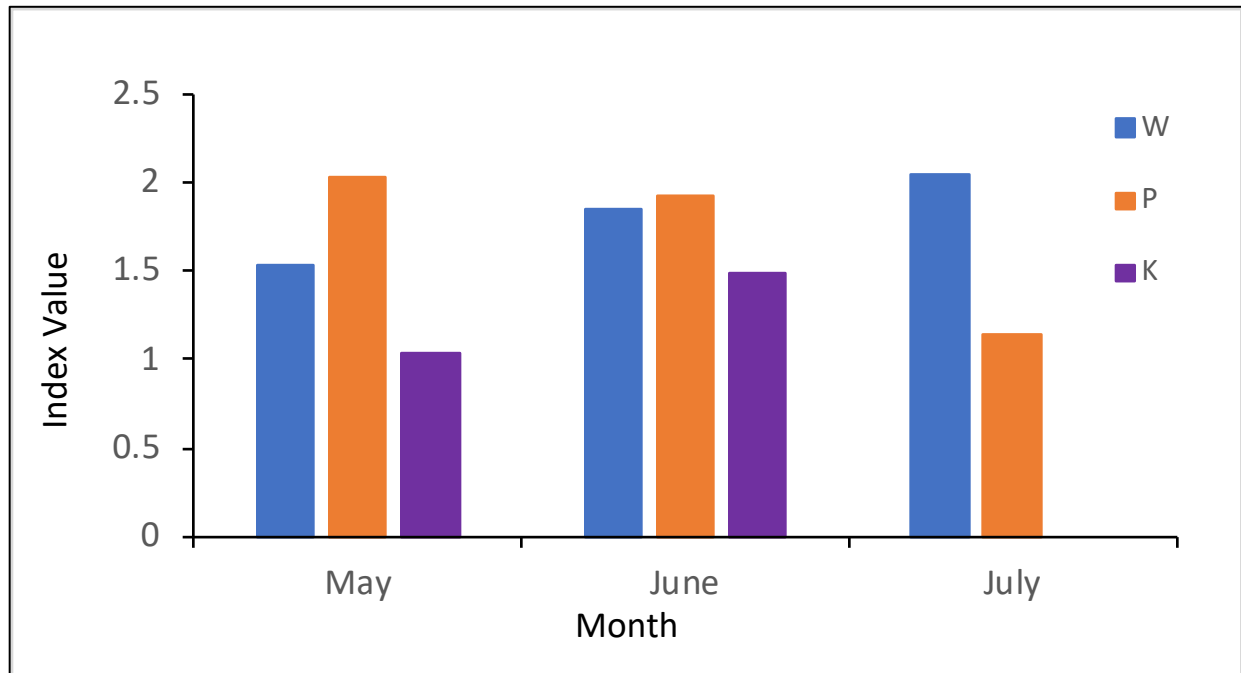
**Figure 1.13.** Weld County diversity for bee genera with five or more individuals.



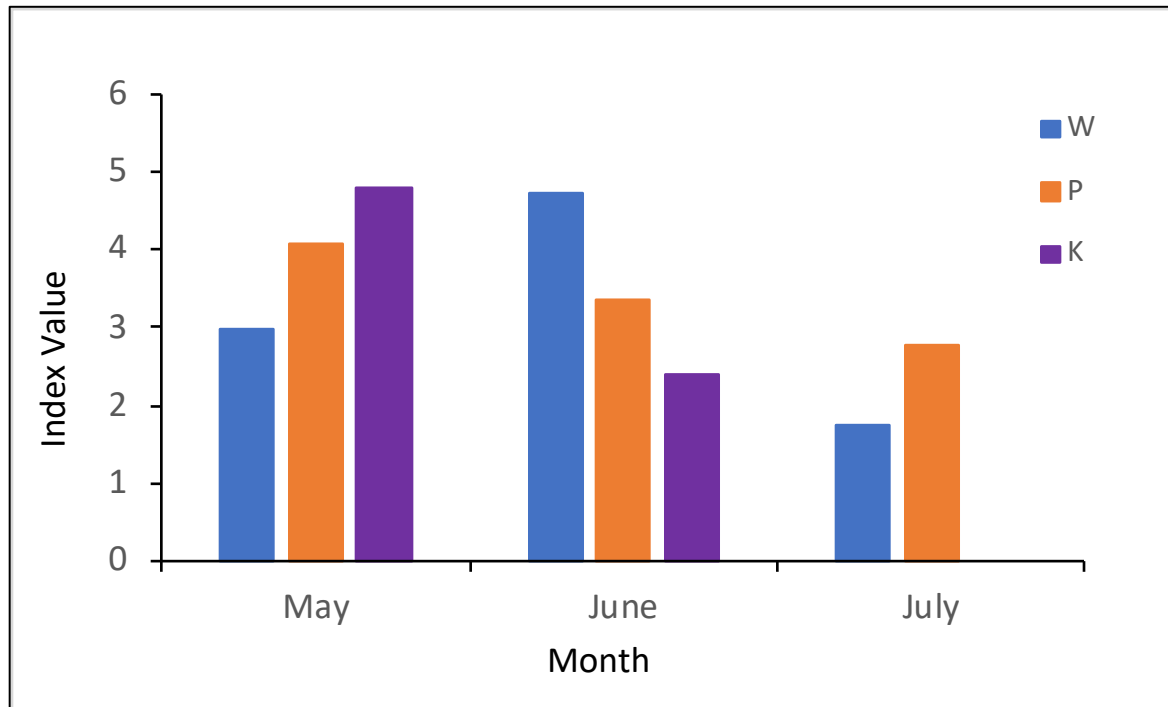
**Figure 1.14.** Weld County diversity for bee genera with five or more individuals when *Lasioglossum* excluded.



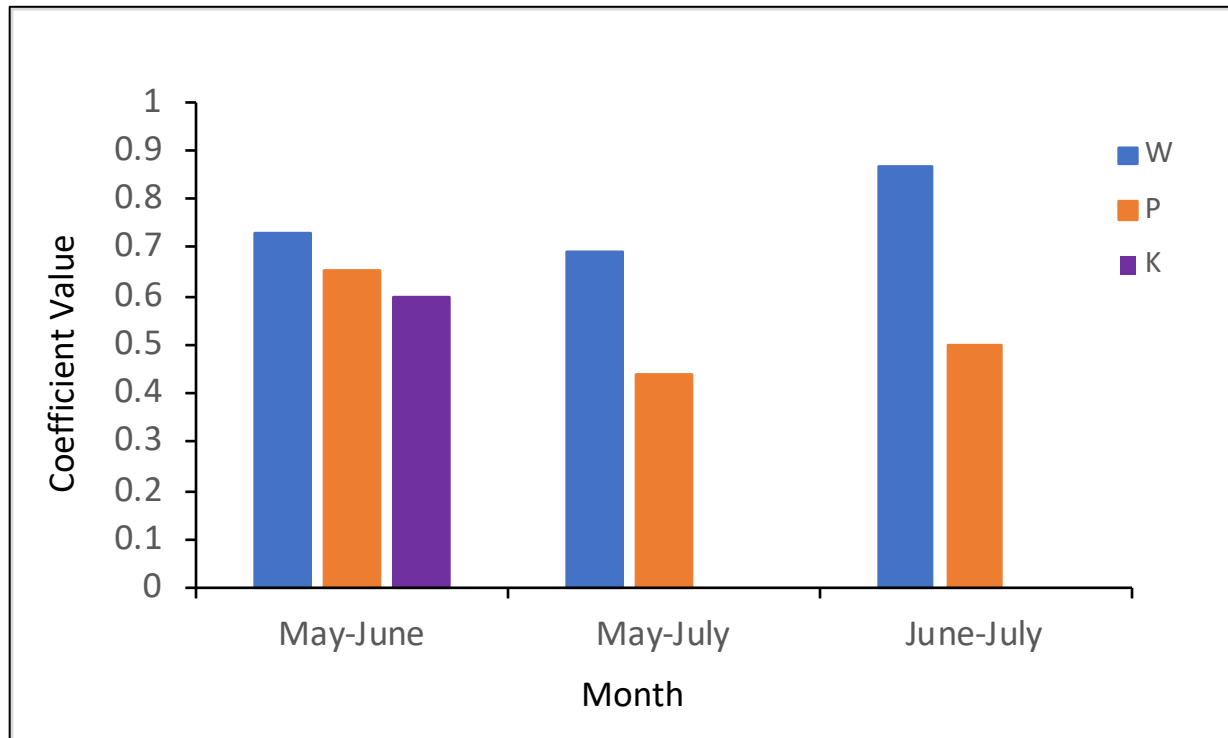
**Figure 1.15.** Shannon-Weiner index values for Weld County, Colorado (W), Perkins County, Nebraska (P), and Kit Carson County, Colorado (K).



**Figure 1.16.** Simpson index values for Weld County, Colorado (W), Perkins County, Nebraska (P), and Kit Carson County, Colorado (K).



**Figure 1.17.** Sorenson's coefficient for Weld County, Colorado (W), Perkins County, Nebraska (P), and Kit Carson County, Colorado (K).



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## Chapter two

### Evaluating the Role of Beekeeping Education and Management on *Varroa* Mite Loads and Hive Survival in Colorado.

#### Introduction

The western honey bee or European honey bee (*Apis mellifera* L.) has long been prized for producing wax and honey as well as being the major pollinators of agricultural crops (Southwick and Southwick 1992; vanEngelsdorp and Meixner 2010; Crittenden, 2011; Hung et al., 2018). In North America, it has been estimated that \$16-\$20 billion dollars of crops benefit directly or indirectly from pollination services provided by honey bees (Gallai et al., 2009; vanEngelsdorp and Meixner 2010; Calderone 2012). Commercial pollination services in the US are primarily provided by professional beekeepers with operations of over 500 hives where the primary revenue source is the hive rental cost paid by the orchard or crop growers (vanEngelsdorp et al., 2012). The majority of U.S beekeepers however maintain hives in backyards, generally managing fewer than 50 hives (vanEngelsdorp et al., 2012; Kulhanek et al., 2017; Thoms et al., 2019).

Nationally, beekeeping has continued to become a popular backyard hobby for several reasons. Whereas some attraction to the hobby undoubtedly comes from the value of both the honey and wax produced by honey bees, other reasons backyard beekeeping has gained in popularity may be rooted in less obvious, sociocultural motivations (Spivak et al., 2011; Phillips 2014; Andrews 2019). Public interest in honey bees grew in the late 2000s, as part of a national response to alarming colony losses of 30%-90% following the first reporting of Colony Collapse Disorder (CCD) (vanEngelsdorp et al. 2008; Ellis et al., 2010; Spivak 2011). The agricultural and

ecological importance of honey bees has since become widely publicized and has inspired many citizens to engage in backyard beekeeping

Despite this increase in beekeeping, average annual colony losses continue to remain around 35%-40% (Lee et al., 2015, Kulhanek et al., 2017). Whereas no single identified cause is apparently responsible for honey bee losses, several factors have been implicated including nutritional stress from lack of adequate floral resources, parasites, pathogens, and exposure to agrochemicals (Naug 2009; vanEngelsdorp and Meixner 2010; Spivak et al., 2011; Goulson et al., 2015; Arathi et al., 2018). The effect of these stressors is likely synergistic further complicating hive management (Pohorecka et al., 2014; Horn et al., 2016; Henry et al. 2017; Rortais et al., 2017). Measures to offset environmental stressors and fortify colonies against the threat of parasites and pathogens have become paramount for colony survival thus making the knowledge and experience of the beekeeper in identifying and controlling these stress factors an invaluable resource for colony survival (Brodschneider et al. 2015; Jacques et al. 2017).

Many backyard beekeepers are beginning hobbyists that lack experience and familiarity with the signs and symptoms of poor honey bee health and do not have the training necessary to recognize and control the causative agents responsible (Owen 2017). The ectoparasitic mite, *Varroa destructor* (Anderson and Trueman, 2000) negatively impacts the overwintering success of honey bee colonies. The surveillance and control of this mite is a prime example of a trainable management behavior that hobby beekeepers often lack (Dainat et al., 2012; Owen, 2017). The threats posed by these mites are compounded by the lack of experience with colony management among new beekeepers that further exacerbates colony failure and facilitates mite dispersal through swarming and robbing behaviors, posing a serious risk to infesting

neighboring hives (Rosenkranz 2010; Frey 2011). The inability or unwillingness to treat for *Varroa* highlights the need for beekeeper educational programs that emphasize the importance of regular hive management and the role of the beekeeper in overwintering success. Because basic beekeeping principles come down to the judgment of the beekeeper, trained beekeepers are vital for maintaining healthy colonies and key in reducing colony losses. Inexperience and lack of educational resources have historically been barriers for beekeepers to adopt proven practices for the prevention and control of parasites and disease (Jacques 2017). As such providing beginning beekeepers with a science-based training curriculum that teaches best management practices as well as providing mentorship may be instrumental in instilling good beekeeping habits that will assist beginning beekeepers how to avoid making critical errors early on in their beekeeping undertakings.

Partnering with Colorado State University Extension (<https://extension.colostate.edu/>) and Colorado Department of Agriculture (<https://www.colorado.gov/agmain>), beekeeping classes were conducted for beginning beekeepers. This class provided an opportunity to study the role that education plays in colony management and survival. Additionally, by surveying beekeepers across Colorado that have or have not attended a formal class in beekeeping, this study sets out to evaluate the efficiency of the course in preparing beekeepers for the challenges of hive management. By measuring beekeeper experience, the frequency that beekeepers inspect their hives, whether they attended a course or received mentorship, their rate of infestation of *Varroa* and compare whether or not their hives overwintered we hope to determine the role of beekeeper education in overwintering success.

## Material and Methods

### Inspection and Sampling Protocol 2018

Two colonies were randomly chosen for inspection and sampling at each bee yard. Hive tools, gloves, and a paint scraping razor used for cutting out comb for taking a brood sample, were sterilized prior to inspection using either bleach or 70% ethanol and then scrubbed with hot water and pumice soap to ensure the removal of wax or propolis

Inspections followed the beekeeping sample form (Supplement 1) For the purposes of the study, a hive was defined as any beekeeping structure (Langstroth hive body, Warre hive body, top-bar hive body, etc.) that housed a single colony. Live bees and brood were sampled from both the hives.

**Brood sampling:** For each colony, approximately 5cm<sup>2</sup> of healthy brood comb was cut or scraped out of the frame to test for the presence of Nosema, American and European foul brood pathogens. A different hive with healthy brood was sampled when necessary. Comb samples from both the sample colonies were then placed into a single Kroger Band regular sized brown paper lunch bag purchased from Walmart and labeled with the sample number corresponding to the beekeeper.

**Live bee sampling:** A frame was removed and inspected to ensure that the queen was not present. Approximately 60 milliliters (~150 bees per hive) were taken from the frame and transferred to a sample bottle about half full of 70% ethanol. Once the bees were dead, excess alcohol was drained and discarded. The bottle was labeled with a sample number that corresponded to a beekeeper and year. The samples with the sample form and questionnaire were sent to the Colorado Department of Agriculture (CDA) bimonthly to be mailed to Bee

Research Laboratory (BRL) at the Beltsville Agricultural Research Center in Beltsville, Maryland,  
for testing

COLORADO DEPARTMENT OF AGRICULTURE  
Division of Plant Industry  
305 Interlocken Pkwy,  
Broomfield, CO 80021

Bee Disease Diagnosis  
Bee Research Laboratory  
10300 Baltimore Ave. BARC-East  
Bldg. 306 Room 316  
Beltsville Agricultural Research Center - East  
Beltsville, MD 20705

For a step-by-step protocol see supplement 3.

### **Sampling protocol 2019**

Before beginning the 2019 Summer surveys, the 2018 questionnaires and procedures were evaluated and the following changes were made: a revised beekeeper questionnaire (Supplement 2, section 2.2); a separate inspector questionnaire (Supplement 2, section 2.3); a GPS location of the bee yards; and *Varroa* sampling was done on site as well as samples sent to the USDA Bee Research Laboratory (BRL). Figure 2.1 shows the locations and inspection year of beekeepers in 2019 and 2018.

**Factors determining hive survival for the purpose of study are described below:**

#### **1. Hive management**

Hive management was determined through the use of surveys. Surveys included an inspection when a sample form (Supplement 1) was filled out with the assistance of the beekeeper and a questionnaire was provided to be filled out by the beekeeper (Supplement 2, section 2.1).



Routine management was given a score of zero in the logistic regression model for beekeepers that inspected their hives fewer than once per month.

## **2. Beekeeper education**

Questions pertaining to beekeeper education and numbers of years spent beekeeping were included within the beekeeper questionnaire (Supplement 2, section 2.1 and Supplement 2, section 2.2). If beekeepers indicated that they had attended a class or received mentorship then that beekeeper was considered to have received a beekeeping education. A beekeeper was considered to have undergone beekeeper education if they either had received mentoring or if they had attended an in-person beekeeping class. Online materials or courses were not considered to meet the criteria.

## **3. Beekeeping experience:** Participating beekeepers were categorized based on the number of years they maintained bee colonies

- Beekeepers with experience of five or less years
- Beekeepers with more than five years-experience but less than 15 years
- Beekeepers with more than 15 years-experience but less than 25 years

**4. *Varroa* mite load:** *Varroa* mite load was determined for each apiary by taking a composite sample from two hives. Bee samples were taken by shaking a brood frame collected from each hive into a sterilized Tupperware bin so that a total of 120 mL of bees (~300 bees) were collected. The bees were then transferred to a 0.5 L jar sealed with a double-sided mesh lid for accommodating two jars. Alcohol was added to the jar to cover the entire sample of bees. The second jar was then fitted to the other lid and the two jars

were vigorously shaken for 90 seconds before filtering the mites into one of the jars. Mites in the alcohol were counted to obtain mite load. The entire process was repeated to ensure count accuracy (Walker et al., 2014; Seshadri and Walker 2019).

Varroa infestation was calculated as follows: The number of mites divided by the number of adult bees (~300) multiplied by 100. This sampling procedure is reviewed in the procedures presented in the extension course (Supplement 4). *Varroa* presence was considered positive when hives had mite infestation rates of 3% or higher, the recommended treatment threshold (Lee et al. 2010).

### **The role of the previous four factors on hive survival**

The role of the previous factors on hive survival and over wintering success was determined by contacting beekeepers during winter and early spring requesting data for the number of hives surviving successfully after the winter in 2019 and 2020. This number was compared to the number of hives the beekeepers started with in 2018 and 2019 respectively (Supplement 2, Section 2.4, Supplement 2, section 2.5., and Supplement 2, section 2.6).

### **Timeline of the study**

#### **Summer 2018**

Beginning in June, invitations were emailed to beekeepers throughout Colorado requesting their participation in the 2018 beekeeping survey (Supplement 5). Participating beekeepers could be of any experience level. Contact information was collected by the Colorado Department of Agriculture or were found through interested beekeeping clubs statewide. A small portion of the sampled beekeepers became aware of the survey through word of mouth and provided their contact information through other beekeepers that had already completed

the survey. All the beekeepers managed bees at a backyard level ( $< 50$  colonies), with the majority of participants having 10 or fewer colonies, except one participant with 11 colonies and another with 35 colonies. Ideally all beekeepers had at least two colonies that could be sampled however there were six cases where the beekeeper had only one colony available and were still allowed to participate. A total of 28 beekeepers were surveyed between June 2018 and September 2018.

### **Winter 2018-Spring 2019**

Over the winter and spring (December 2018-May 2019) cooperator beekeepers participating in the study were contacted to determine hive survival and any additional management practices that may have occurred after the 2018 summer surveys (Supplement 2, Section 2.4 and Supplement 2, section 2.5). At this time responses were received from 64% of the beekeepers sampled. During the spring beekeepers were again invited to participate in the 2019 summer surveys.

### **Summer 2019**

Summer surveys were conducted following the sampling protocol for 2019. Twenty-two beekeepers participated in the 2019 survey; half were returning 2018 participants while the others were contacted using contact information obtained either from a sign-up sheet distributed during a Colorado State University (CSU) extension course or through existing contacts.

### **Spring 2020**

Data acquisition was finalized in the Spring of 2020 by contacting the beekeeper participants regarding any additional management they had performed on their hives post inspection and

their overwintering success. Of the 23 responses, 65% came from returning participants. Two participants from 2018 retroactively provided hive survival for 2019 at this time despite having not actively participated in 2019 inspection.

## **Analysis**

A summary analysis of beekeeper experience was done. There were 41 responses gathered from Spring 2018 to Spring 2020 that were used in the analysis. The criteria for successful hive management was considered for any beekeeper who performed regular hive inspections and management including treating for pathogens, feeding, and adding additional hive bodies/supers as needed.

Beekeeper experience categories:

1. Beekeepers with experience of five or less years (22 participants)
2. Beekeepers with more than five years-experience but less than 15 years (16 participants)
3. Beekeepers with more than 15 years-experience but less than 25 years (3 participants).

For these three categories, the following values were calculated. Proportions of

1. Successfully overwintered hives
2. Beekeepers performing routine hive management
3. Beekeepers receiving mentoring/training
4. Hives with mite infestation rate greater than 3%

Wilcoxon Signed Ranks tests were performed using IBM SPSS Statistics v. 26.to determine if training received by beekeepers affected the *Varroa* load and hive survival in their apiaries.

Survival data was collected during winter and spring follow up questionnaires while *Varroa* data was collected during the summer hive inspections.

*Varroa* mite load comparison training categories:

1. CSU training (9 participants)
2. Bee club training (5 participants)
3. No training (12 participants)
4. Undisclosed training (19 participants)
5. Other training (5 participants)

Hive survival comparison training categories

1. CSU training (4 participants)
2. Bee club training (6 participants)
3. No training (2 participants)
4. Undisclosed training (8 participants)
5. Other training (6 participants)

Wilcoxon Signed Ranks test was performed to determine differences in hive survival in relation to training received.

1. Trained beekeepers (16 participants)
2. Untrained beekeepers (10 participants)

## **Results**

### **Hive management**

Beekeepers with <25 years of experience and beekeepers with <5 years of experience performed routine hive management more than beekeepers with less than <15 years of experience in both 2018 and 2019. The proportion of beekeepers that performed routine hive management did not change from 2018 to 2019 for beekeepers with <25 years of experience and beekeepers with <15 years of experience but there was a slight increase in hive management for the beekeepers with <5 years of experience (Figure 2.2).

### **Beekeeper education**

In 2018 and in 2019 all the beekeepers with <25 years of experience and beekeepers with <15 years of experience were educated while ~78% of beekeepers with <5 years of experience were educated in 2018 with a small decrease in 2019 to 77% (Figure 2.3).

### **Mite load during inspection**

Beekeepers from the <25 years of experience group in 2018 were the lowest proportion (0 beekeepers) with mites loads at the 3% threshold but in 2019 were the highest proportion with mites at the same infestation threshold. In both 2018 and 2019, beekeepers in the <5 years of experience group were the lowest proportion with mite infestations at the 3% threshold. In 2018, beekeepers with <15 years of experience were the highest proportion with mite infestations at the 3% threshold and in 2019 were the second highest proportion at the same threshold (Figure 2.4).

### **Training and *Varroa* observations**

The average mite load observed during hive inspections in apiaries of beekeepers that received training from CSU (n=9) was  $1.62 \pm 2.06$  mites per 100 bees (Table 2.1). The average mite load in apiaries of beekeepers receiving training from a bee club (n=5) was  $0.47 \pm 0.56$  mites per 100 bees. The colonies in the apiaries of beekeepers that had no formal training (n=12) had an average of  $2.31 \pm 3.24$  mites per 100 bees. The colonies in the apiaries of beekeepers that indicated “other” for training (n=5) had  $5.00 \pm 4.67$  mites per 100 bees. The colonies in the apiaries of beekeepers that did not disclose details on training (n=19) had  $2.38 \text{ mites} \pm 2.80$  per 100 bees (Table 2.1).

There was no significant difference in the mite load of colonies in the apiaries of beekeepers that received training at CSU and those in the apiaries of beekeepers that received training at a bee club. Similarly, there were no significant differences in the mite load of colonies in the apiaries of beekeepers that indicated “other” for training, from colonies in apiaries of beekeepers that received no formal training, and those in apiaries of beekeepers whose training was undisclosed (Table 2.2). While not significant, the data was trending for a difference in mite load in apiaries of beekeepers that received other training compared to the apiaries of beekeepers that attended the CSU beekeeping course ( $p = 0.068$ ) as well as in apiaries of beekeepers with “other” training when compared to apiaries of beekeepers with the bee club training ( $p = 0.080$ ). When compared to those that received training from a bee club, apiaries of beekeepers that did not disclose their training showed no significant difference in mite load. There was no significant difference in mite load between the apiaries of beekeepers with undisclosed training and that of beekeepers with no formal training (Table 2.2).

## **Hive survival**

Average hive survival was greatest for beekeepers with <15 years of experience for the 2018 beekeeping year but this group had the lowest 2019 average hive survival although the number of beekeepers in this category did not change between years. For 2018-2019, the average hive survival was similar for beekeepers with less than <25 years of experience and those with <5 years of experience. For 2019-2020 the beekeepers with <25 years of experience had the highest average hive survival and beekeepers with <5 years of experience followed as the second highest average hive survival. Both groups had an increase in number of beekeepers surveyed (Figure 2.5). Average hive survival increased for all three categories from the 2018 to the 2019 beekeeping year. For the 13 returning beekeeper responses, nine had improved hive survival (Table 2.3).

## **Training and hive survival**

The average proportion of hives that survived in the apiaries of beekeepers that received training from CSU (n=4) was  $71\% \pm 48\%$  (Table 2.4). Hive survival in the apiaries of beekeepers that received training from a bee club (n=6) was  $67\% \pm 41\%$ . Hive survival in the apiaries of beekeepers that received training labeled “other” (n=6) was  $63\% \pm 34\%$ . The apiaries of beekeepers that did not have any formal training (n=2) had  $100\% \pm 0\%$  and apiaries of those with undisclosed training (n=8) had a hive survival of  $62\% \pm 42\%$ .

Re-calculating the average by grouping the data into one category for trained beekeepers (n=16), hive survival was  $66\% \pm 38\%$ . Re-calculating the average for untrained beekeepers (i.e. beekeepers with no training and beekeepers with undisclosed training; n=10) hive survival was  $70\% \pm 40\%$  (Table 2.3). There was no difference in the proportions of hives



that survived across all training groups when compared to the proportion of hives that survived in apiaries of beekeepers that received training at CSU (Table 2.5). There was also no significant difference in hive survival across all training types when compared to the bee club training. There was no significant difference between undisclosed training and no formal training. When comparing the trained beekeepers to untrained beekeepers there was no significant difference in hive survival (Table 2.5).

### **Discussion**

Beekeepers with less than 25 years of experience and beekeepers with less than five years of experience included a greater proportion of beekeepers that undertook routine hive management and had better hive survival regardless of mite load. This was expected for beekeepers with less than 25 years of experience but was less likely from relatively new beekeepers with less than five years of experience. It was also unexpected that beekeepers with less than 15 years of experience would perform well in 2018 despite a lower proportion of them undertaking routine hive management. While it is not clear from my study why a higher number of the beekeepers with less than five years of experience performed routine hive management than met the education criteria., It may be that the education criteria established by the study did not encompass alternative educational resources available to beekeepers and how these alternative educational resources influenced management. With regard to high hive survival in 2018 among beekeepers with less than 15 years of experience, even though fewer proportion of them performed routine hive management, a monthly hive inspection regime may have been too stringent of a criterion to determine its effect on hive survival.

## **Hive management**

In continuation, overly frequent hive monitoring may be more intrusive than otherwise assumed. It may be reasonable to inspect every six weeks or three times throughout the season. Beekeeper intervention *via* inspection and management prior to overwintering, particular for *Varroa* mite control is critical. This is supported by previous research (Dainat et al. 2012; Döke et al., 2015). Given the way in which hive development progresses in Colorado (*personal observations*), hive inspections in June and August for *Varroa* mites is necessary (Arathi and Walker 2020). In addition to disease management, promoting strong bee populations in hives with greater number of individuals and good honey stores are important for overwintering success (Döke et al., 2015). Thus, supplemental feeding that promotes brood production and hive strength early in the season and mid-winter when forage is unavailable are encouraged to be a routine practice.

## **Influence of training on hive survival**

While there was no significant difference in hive survival across the different beekeeping training groups this may have been a result of a low sample size. As mentioned above, hive survival can be increased through simple and effective hive management practices and the CSU training and bee club training emphasized what hive management entails throughout a beekeeping year. A study with a larger sample size could potentially see a difference in hive survival for the different training types.

## **Beekeeper education**

The criteria used for beekeeping education in this study was attending a course or receiving mentoring but expanding the criteria to include books or extension resources accessed online

might be worth considering in order to explain the observed pattern of results in the study.

Accessibility to print media or online text may have similar impact on hive survival compared to in person mentoring or a beekeeping course as long as the resources promote good beekeeping practices like hive management which hive survival ultimately depends on (Jacques et al., 2017; Döke et al., 2015). With an abundance of online material, access to reliable online websites has become increasingly important. To help ensure scientific accuracy, extension agents could provide a list of reliable websites and print materials when contacted by beekeepers or while conducting hive inspections.

### **Influence of training on mite load**

Training was categorized into five groups depending on where training was received. There was no significant difference in *Varroa* mite load between any of the beekeeping training groups, the data was trending to be more significant for the CSU training compared to other training. The data was also trending to be more significant for the bee club training compared to other training. The bee club training was similar to the CSU training and so it is possible that with a larger sample size of beekeepers, there may be a significant effect of training on mite load.

*Varroa* mites are ectoparasites that feed on bee hemolymph and negatively impact bee health and physiological development (Döke et al., 2013). High mite loads often lead to the death of a colony but timely hive management can prevent this (Rosenkranz et al., 2010). All the beekeepers from the study performed routine hive management suggesting that mite load at the time of the inspection was within the recommended levels for overwintering hives. When mite loads exceed the threshold in Fall, treatment must be applied to prevent winter losses (Currie and Gatien, 2006; Dainat et al. 2012; Döke et al., 2015). It is recommended that

beekeepers treat in spring to prevent mite build up for late summer and to treat again in the fall to prevent winter losses when mite loads exceed the treatment threshold. These times in Colorado match with the June and Aug-Sep hive inspections mentioned above, thus making these important times in the season for mite sampling (Currie and Gatien, 2006). All the beekeepers with less than 25 years of experience performed routine hive management including mite surveillance and beekeeper intervention for reducing mite loads, an important step for winter hive survival (Currie and Gatien, 2006; Rosenkranz et al., 2010; Dainat et al. 2012; Döke et al., 2015).

### **Experience**

It is reasonable that beekeepers with less experience would be less capable of identifying and controlling issues within the hive and maintaining bee yard sanitation to reduce pathogen spread (Sporandio et al., 2019). Regardless of experience, good practices and husbandry are what ultimately determine hive survival. Exposure to reliable beekeeping resources that promote good practices and husbandry, is not necessarily a result of time spent beekeeping but rather may reflect the level of beekeeper commitment to learning and establishing good beekeeping practices.

## Tables

<b>Table 2.1.</b> <i>Varroa</i> mite loads in apiaries of beekeepers by training.					
Training category	n	Mean	Std. Deviation	Minimum	Maximum
CSU training	9	1.63	2.06	0.00	6.00
Bee club training	5	0.47	0.56	0.00	1.33
No training	12	2.31	3.24	0.00	9.00
Undisclosed training	19	2.38	2.80	0.00	8.40
Other	5	5.00	4.67	0.00	11.67

<b>Table 2.2.</b> The results from the Wilcoxon signed ranks test comparing differences in <i>Varroa</i> mite loads in apiaries of beekeepers across the different beekeeping training categories.					
Training (Z, p)	CSU	Bee club	Undisclosed	Other	No training
CSU	x	x	x	x	X
Bee Club	-1.07, 0.29	x	x	x	X
Undisclosed	-0.84, 0.4	-0.94, 0.35	x	X	X
Other	-1.83, 0.07	-1.75, 0.08		X	X
No training	-0.56, 0.58	-1.08, 0.3	-0.63, 0.5		X

**Table 2.3.** Hive survival (%) in sampling years, 2018 and 2019 for apiaries of beekeepers with different experience, Routine Hive Management (RHM) and education levels

Beekeeper ID	2018	2019	Experience	RHM 2018	RHM 2019	Education (mentoring or training)	Survival Comparison
BK1	0.60	0.7	<15	no	no	yes	improved
BK2	0.00	1	<5	yes	yes	no	improved
BK3	0.00	1	<15	yes	yes	yes	improved
BK4	1.00	0.6	<15	no	no	yes	worse
BK5	0.00	1	<15	yes	yes	yes	improved
BK6	0.50	1	<5	yes	yes	yes	improved
BK7	1.00	1	<5	yes	yes	yes	no change
BK8	0.86	1	<5	yes	yes	yes	improved
BK9	0.50	1	<25	yes	yes	yes	improved
BK10	0.00	0.75	<15	yes	yes	yes	improved
BK11	1.00	0	<15	yes	yes	yes	worse
BK12	0.83	0.57	<15	yes	yes	yes	worse
BK13	0.67	1	<5	yes	yes	no	improved

<b>Table 2.4.</b> Proportion of hives that survived in the apiaries of beekeepers in the different training categories.					
Training category	n	Mean	Std. Deviation	Minimum	Maximum
CSU training	4	0.71	0.48	0.00	1.00
Bee club training	6	0.67	0.41	0.00	1.00
No training	2	1.00	0.00	1.00	1.00
Undisclosed training	8	0.62	0.42	0.00	1.00
Other	6	0.62	0.34	0.00	1.00
Trained beekeepers*	16	0.66	0.38	0.00	1.00
Untrained beekeepers**	10	0.70	0.40	0.00	1.00
*: Trained beekeepers = CSU training + Bee Club Training + Undisclosed training					
**: Untrained beekeepers = no training + undisclosed training					



**Table 2.5.** The results from the Wilcoxon signed ranks test comparing hive survival for beekeepers in the different beekeeping training categories.

Training (Z, p)	CSU	Bee club	Undisclosed	Other	No training
CSU	x	x	x	x	X
Bee Club	-0.45, 0.65	x	x	x	X
Undisclosed	-0.45 ,0.65	-0.54,0.59	x	X	X
Other	0.00,1.00	-0.54,0.60		X	X
No training	0.00,1.00	0.00,1.00	-1.00,0.32	-1.34,0.18	X

Trained\* Vs Untrained beekeepers\*\*: -0.21, 0.83

\*: Trained beekeepers = CSU training + Bee Club Training + Undisclosed training

\*\*: Untrained beekeepers = no training + undisclosed training

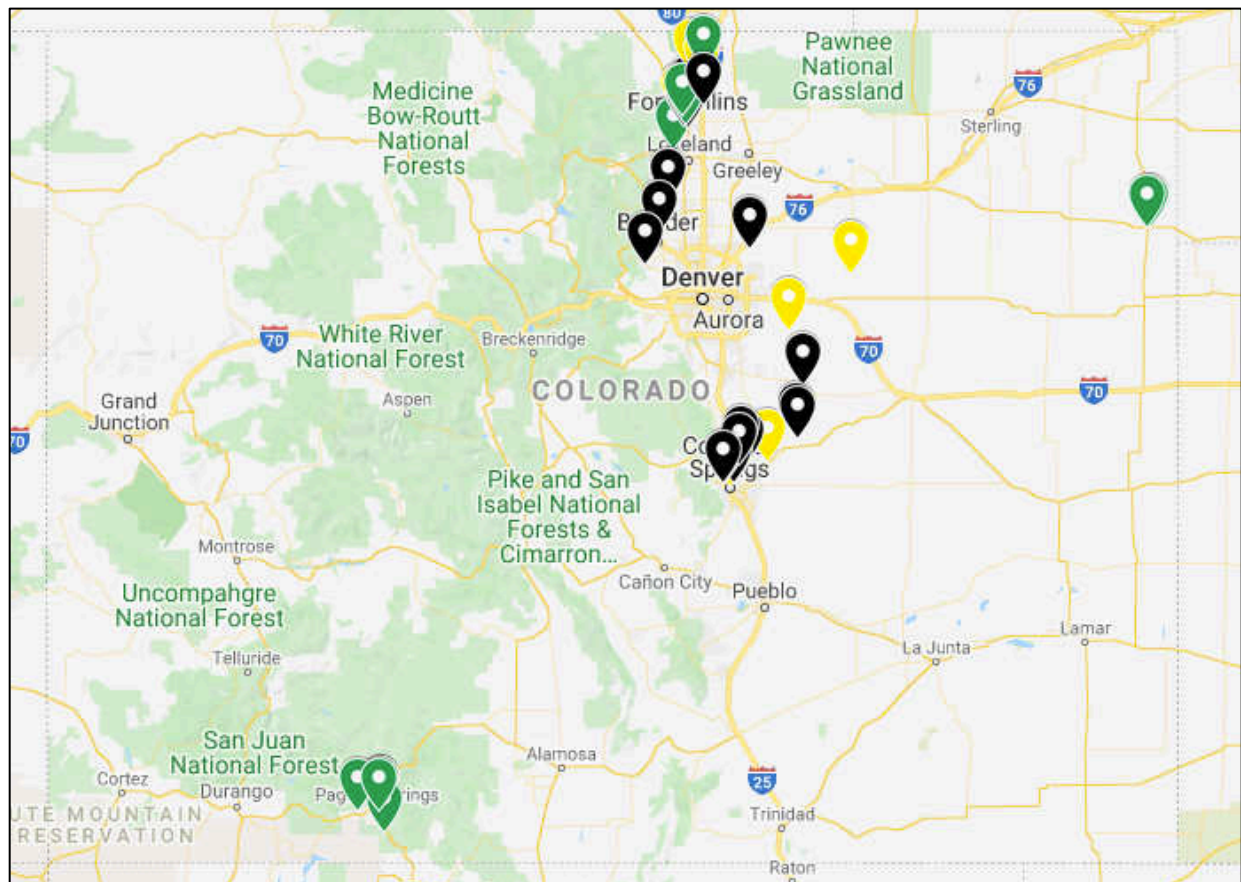
## Figures

**Figure 2.1.** The location of bee yards sampled for this study across the state of Colorado.

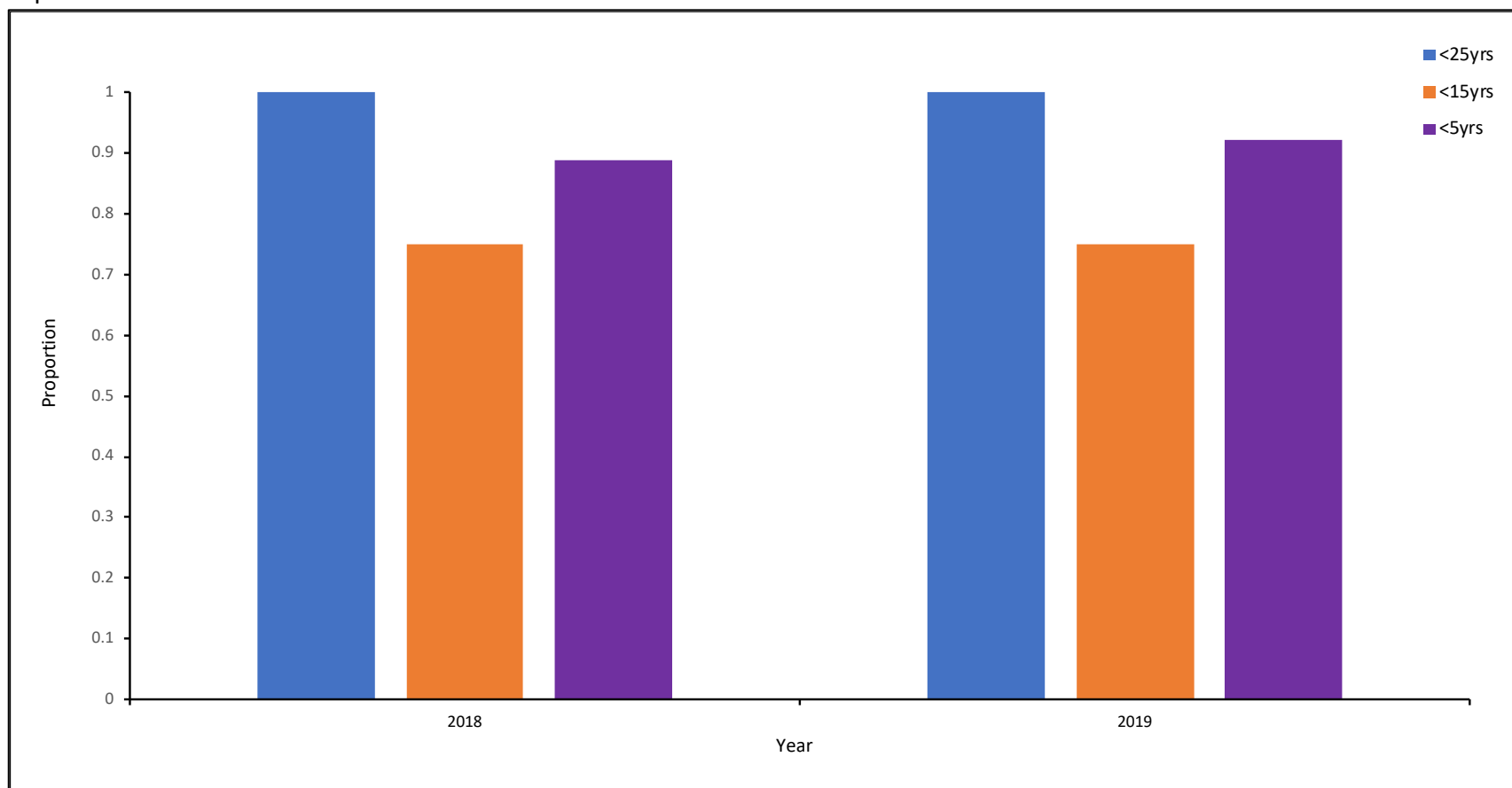
Yellow pins (11): Beekeepers that participated in 2018 and 2019

Green points (11): 2019 participants

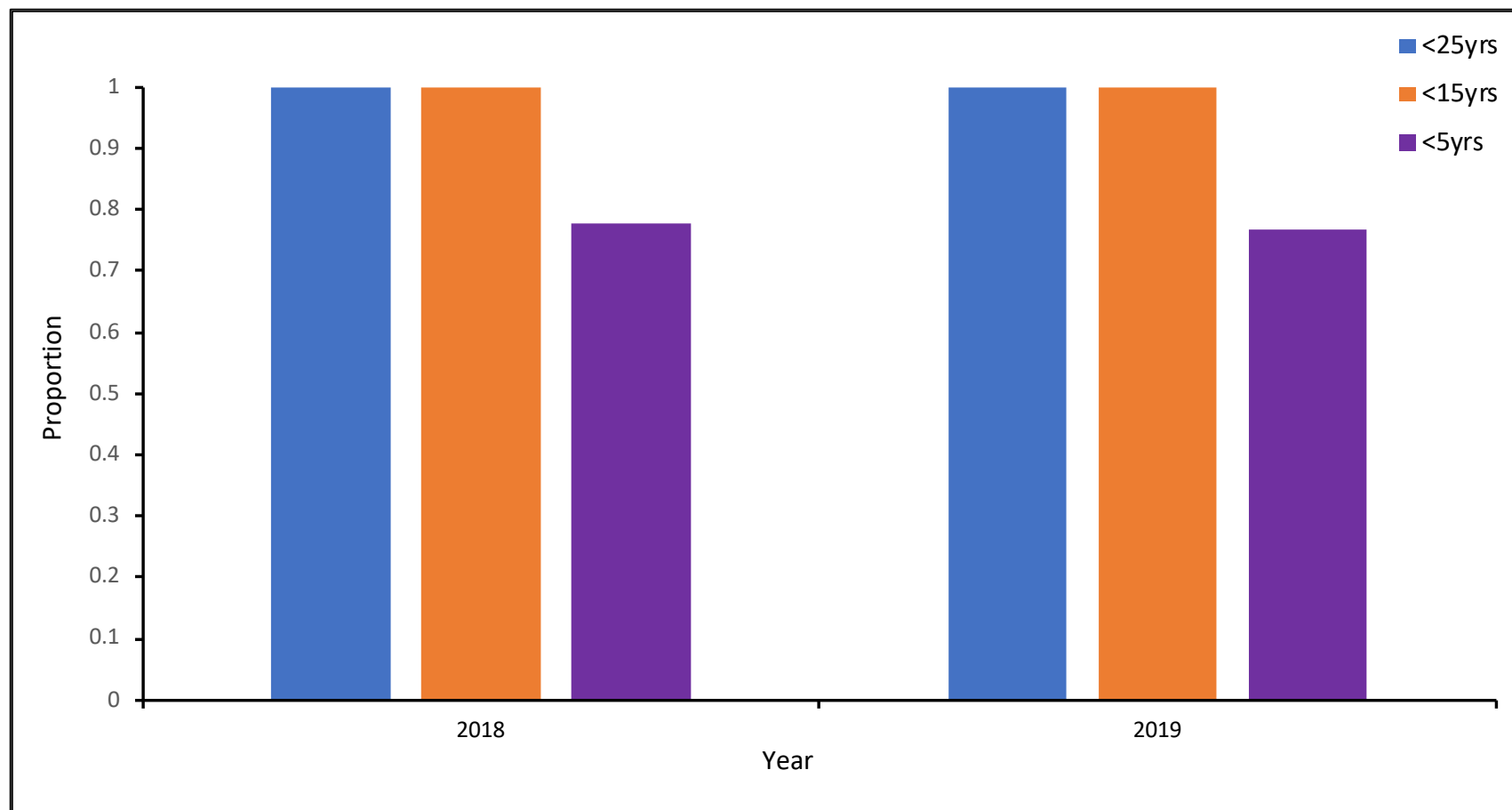
Black points (17): 2018 participants.



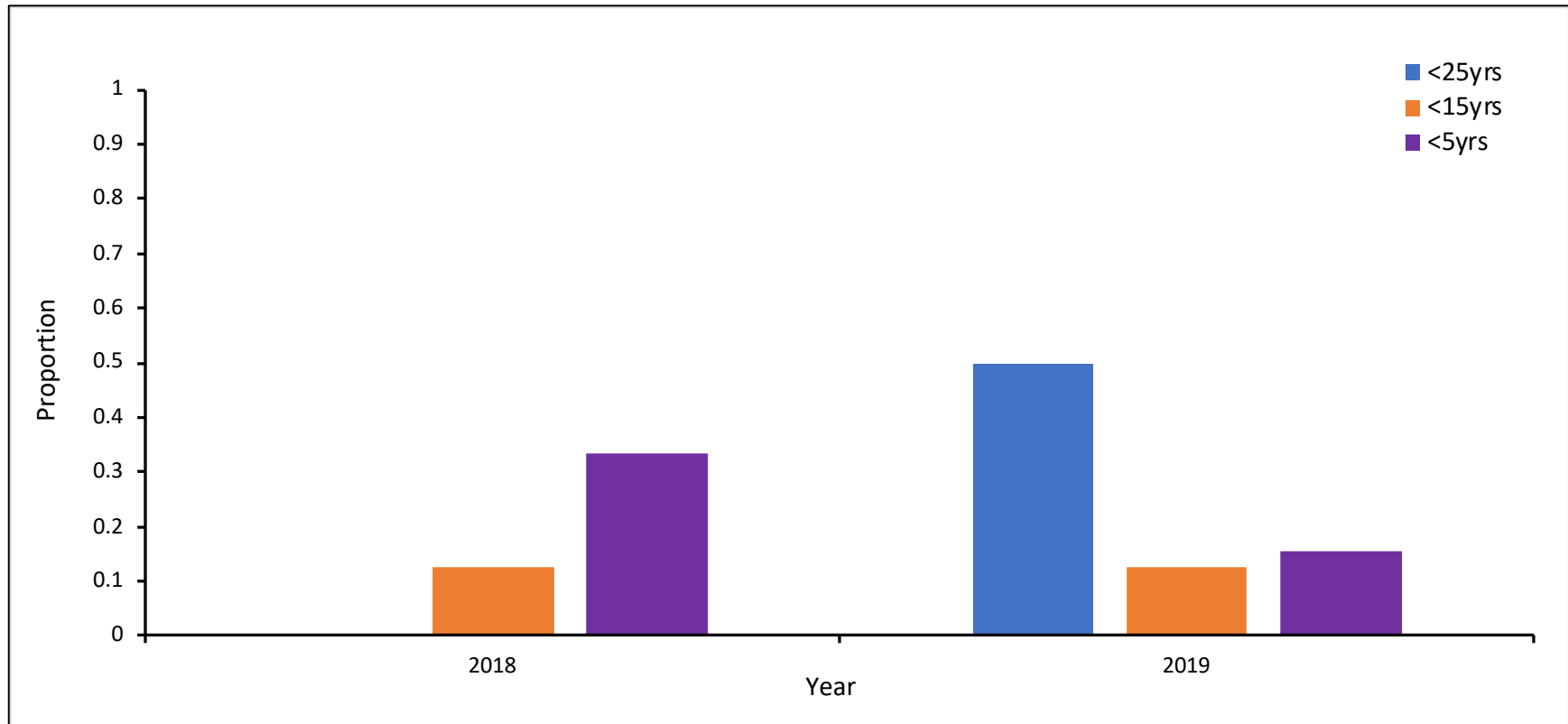
**Figure 2.2.** The proportion of beekeepers that performed routine hive management in 2018 and 2019 grouped by years of experience.



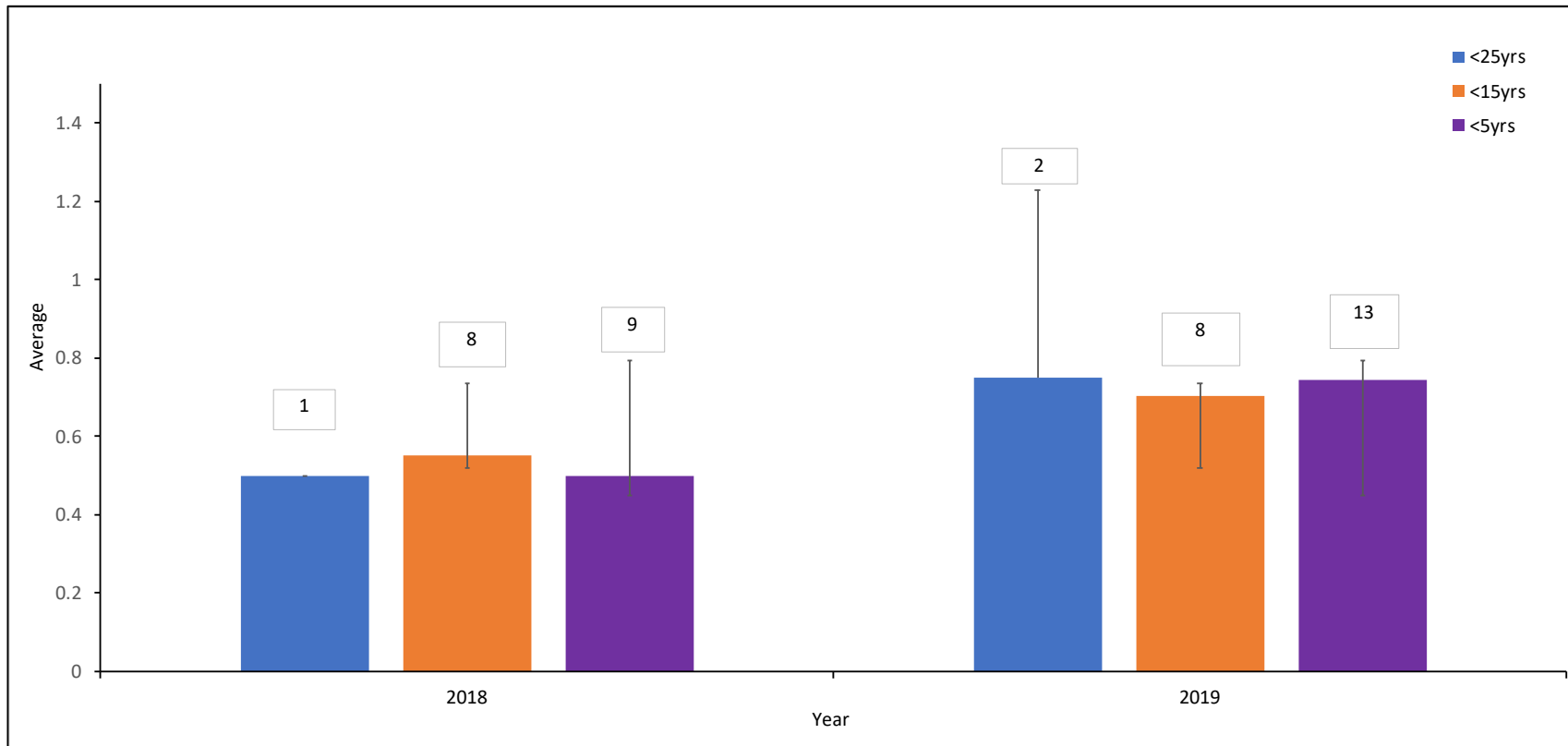
**Figure 2.3.** The proportion of beekeepers that had a beekeeping education (mentoring or training) in 2018 and 2019 grouped by years of experience with beekeeping.



**Figure 2.4.** The proportion of beekeepers with mite loads  $\geq 3\%$  threshold in apiaries of beekeepers with different years of experience with beekeeping. In 2018, no beekeeper with  $>25$  years of beekeeping experience had mite loads above the 3% threshold.



**Figure 2.5.** The average ( $\pm$  SD) hive survival for beekeepers grouped by years of experience. The number of beekeepers is indicated above each experience category.



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## **Appendices**

Supplement 1: 2018 Sample form

**2018 Sample Form**

COLORADO DEPARTMENT OF AGRICULTURE  
Division of Plant Industry  
305 Interlocken Pkwy.  
Broomfield, CO 80021  
(303) 869-9050  
**BEE SAMPLE FORM**

Name of beekeeper:

Address:

Phone No.:

Email:

Name of landowner:

Address:

Phone No.:

Name and location of Bee yard if different from above:

Number of colonies in Bee yard:

Number of colonies sampled:

Name of Sampler

Sample Number

Were samples taken from this hive (commodity & sample number):

**HIVE NUMBER** 1

**CONDITION OF HIVE**

How many supers? \_\_\_\_\_

Frames per super? \_\_\_\_\_

How many frames observed? \_\_\_\_\_

Queen observed? \_\_\_\_\_

Eggs:

☐ Yes ☐ No ☐ Multiple

Honey - weight of supers:

☐ light ☐ medium ☐ heavy

Strength of bees (0-5) \_\_\_\_\_ (0=No Bees, 5=Ready to Swarm)

Amount of pollen (0-5) \_\_\_\_\_ (0=No pollen, 5= 2inches around all Brood)

Color of pollen:

☐ yellow ☐ orange ☐ white  
☐ brown ☐ other \_\_\_\_\_

**OBSERVATIONS OF HIVE (diseases, etc.)**

☐ Foul Brood ☐ Aggressive

☐ Varroa Mite ☐ Crawling

☐ Heavy chalk brood ☐ Shivering

☐ K-wing ☐ Spinning

☐ Dead bees on bottom board  
If yes, how much (in pts.)? \_\_\_\_\_

☐ Extender patty ☐ Apistan strips

☐ Menthol ☐ Other \_\_\_\_\_

**HIVE NUMBER** 2

**CONDITION OF HIVE**

How many supers? \_\_\_\_\_

Frames per super? \_\_\_\_\_

How many frames observed? \_\_\_\_\_

Queen observed? \_\_\_\_\_

Eggs:

☐ Yes ☐ No ☐ Multiple

Honey - weight of supers:

☐ light ☐ medium ☐ heavy

Strength of bees (0-5) \_\_\_\_\_ (0=No Bees, 5=Ready to Swarm)

Amount of pollen (0-5) \_\_\_\_\_ (0=No pollen, 5=2 inches around all Brood)

Color of pollen:

☐ yellow ☐ orange ☐ white  
☐ brown ☐ other \_\_\_\_\_

**OBSERVATIONS OF HIVE (diseases, etc.)**

☐ Foul Brood

☐ Aggressive

☐ Varroa Mite

☐ Crawling

☐ Heavy chalk brood

☐ Shivering

☐ K-wing

☐ Spinning

☐ Dead bees on bottom board

If yes, how much (in pts.)? \_\_\_\_\_

☐ Extender patty

☐ Apistan strips

☐ Menthol

☐ Other \_\_\_\_\_

Were samples taken from this hive (commodity & sample number): \_\_\_\_\_

BRL please return results to Laura Pottorff at the Colorado Dept. of Ag. and the above listed Bee Keeper.

Supplement 2: Questionnaire

**Supplement 2.1. 2018 Beekeeping Questionnaire**

COLORADO DEPARTMENT OF AGRICULTURE  
Division of Plant Industry  
305 Interlocken Pkwy.  
Broomfield, CO 80021  
(303) 869-9050  
Fax: (303) 466-2860

**BEEKEEPER QUESTIONNAIRE**

Name of beekeeper

Address

Phone No.

Name of landowner

Address

Phone No.

How long have you been a beekeeper?

Are you a member of a beekeeping club or organization? ☐ Yes ☐ No.

If so the name of club or clubs you belong to.

Have you attended a beekeeping class? ☐ Yes ☐ No.

Do you receive mentoring? ☐ Yes ☐ No.

How would you describe your beekeeping type? ☐ Natural ☐ Organic ☐ Conventional ☐

Other \_\_\_\_\_

What type of beehives do you use? ☐ Langstroth ☐ Top Bar ☐ Other \_\_\_\_\_

What was the source of your hives? \_\_\_\_\_

What was the source of your bees? \_\_\_\_\_

What type of bees do you have? \_\_\_\_\_

Have you requeened? ☐ Yes ☐ No and if so when? \_\_\_\_\_

Was queen marked? ☐ Yes ☐ No and if so

how? \_\_\_\_\_

How often to you open and inspect your hives?

\_\_\_\_\_

Total number of colonies \_\_\_\_\_

☐ Nuc ☐ Single queen Number of supers/colony \_\_\_\_\_

Location of bee yard \_\_\_\_\_

Behavior of bees: ☐ Normal ☐ Aggressive ☐ Crawling ☐ Shivering ☐ Spinning; and  
when observed?

\_\_\_\_\_

Has the behavior of the bees stopped, if so when?

\_\_\_\_\_

Weather conditions when behavior observed:

\_\_\_\_\_

Average/Colony (Estimated): Frames of adult bees \_\_\_\_\_ Frames of brood \_\_\_\_\_

Frames of honey \_\_\_\_\_ Square inches of pollen \_\_\_\_\_

How was the brood pattern? ☐ Good (Solid & Uniform) ☐ Mediocre (Intermittent or random)

☐ Poor (Spotty)

Pollen color(s): ☐ Yellow ☐ Orange ☐ White ☐ Brown ☐ \_\_\_\_\_

Feeder present: ☐ Yes ☐ No If Present: ☐ Full ☐ Partial ☐ Empty

Pollen Substitute Used: ☐ Yes ☐ No

Type of feeder

Immature stages present: ☐ Eggs ☐ Young larvae ☐ Old larvae ☐ Pupae

Have mites been noted in colonies?: Varroa mite ☐ Yes ☐ No Tracheal mite ☐ Yes ☐ No

Date and how determined? \_\_\_\_\_

Have any disease problems been noted in the colonies? ☐ Yes ☐ No

If so; what and when? \_\_\_\_\_

Have colonies been treated with chemical/medication: ☐ Yes ☐ No

Product(s) used (dates, how applied, how mixed)

\_\_\_\_\_

Blooming plants within 1-2 miles of apiary/distance and direction



☐ Alfalfa    ☐ Corn    ☐ Sweet clover  
☐ Apple    ☐ Dandelion    ☐ Urban  
☐ Asparagus    ☐ Mint    ☐ Clover  
☐ Mustard    ☐ Other

Additional Comments:

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Signed

## Supplement 2.2. 2019 Beekeeper Questionnaire

### BEEKEEPER QUESTIONNAIRE

Date \_\_\_\_\_

Survey number:

Location: County

How long have you been a beekeeper?

\_\_\_\_\_

Are you a member of a beekeeping club or organization? ☐ Yes ☐ No.

Do you attend meetings ☐ Yes ☐ No

Have you attended a beekeeping class? ☐ Yes ☐ No.

If yes, what class?

\_\_\_\_\_

Do you have a beekeeping mentor: ☐ Yes ☐ No

What other resources do you use for obtaining information regarding beekeeping:

☐ Internet ☐ Books ☐ Additional Classes ☐ Friends ☐ None

How would you describe your beekeeping type (Check all that apply)?

☐ Natural/Treatment Free ☐ Organic ☐ Conventional

What type of beehives do you use? ☐ Langstroth ☐ Top Bar ☐ Warre ☐ Other (please explain)

\_\_\_\_\_

What was the source of your hive

equipment? \_\_\_\_\_

Were your colonies over wintered or obtained new package at the start of the season? \_\_\_\_\_

—

If new, how obtained? ☐ Nuc ☐ Package ☐ Swarm ☐ Other (explain)

\_\_\_\_\_

What was the source of your bees?

☐ Local/Colorado (From Whom)\_\_\_\_\_

☐ Outside of the State (Which State) \_\_\_\_\_

What type of bees do you have (Check all that apply)?

☐ Italian ☐ Carniolan ☐ Russian ☐ Saskatchewan ☐ Hygienic ☐ Other (please explain)\_\_\_\_\_

Have you requeened? ☐ Yes ☐ No and if so when?

\_\_\_\_\_

Source of new queen: ☐ Raise their own ☐ Other (please explain)\_\_\_\_\_

How often do you open and inspect your hives?

☐ Never ☐ Weekly ☐ Bi-Weekly ☐ Monthly

How confident are you that you can recognize disease and abnormalities within your colony?

☐ Not confident ☐ Somewhat confident ☐ Confident ☐ Very Confident

Do you perform routine mite tests ☐ Yes ☐ No

Do you treat your colonies as needed ☐ Yes ☐ No

Have colonies been treated with chemicals/medications: ☐ Yes ☐ No

Product(s) used (dates, how applied, how mixed)

\_\_\_\_\_  
\_\_\_\_\_

Total number of colonies \_\_\_\_\_

Number of hive bodies/colony-\_\_\_\_\_

Number of honey supers

Please list any blooming plants within 1-2 miles of apiary/distance and direction. Also note when they were in bloom

\_\_\_\_\_  
Additional Comments

\_\_\_\_\_

## Supplement 2.3. 2019 Inspector Questionnaire

### Inspector Questionnaire

Date:

Survey Number

Location: County and GPS

Temperature: \_\_\_\_\_

Weather: \_\_\_\_\_

Behavior of bees at inspection: ☐ Normal ☐ Aggressive ☐ K-Wing ☐ Other \_\_\_\_\_

Average/Colony (Estimated): Frames of adult bees \_\_\_\_\_ Frames of  
brood \_\_\_\_\_

Frames of honey \_\_\_\_\_ Frames of Pollen \_\_\_\_\_

How was the brood pattern? ☐ Good (Solid & Uniform) ☐ Mediocre (Intermittent or random)  
☐ Poor (Spotty)

Pollen color(s): ☐ Yellow ☐ Orange ☐ White ☐ Brown ☐ \_\_\_\_\_

Feeder present: ☐ Yes ☐ No If Present: ☐ Full ☐ Partial ☐ Empty

Pollen Substitute Used: ☐ Yes ☐ No

Type of feeder

---

Immature stages present: ☐ Eggs ☐ Young larvae ☐ Old larvae ☐ Pupae

Have Varroa mites been noted in colonies?: Varroa mite ☐ Yes ☐ No

If yes, how many bees per cup of bees (~300 bees)

Date and how determined? \_\_\_\_\_

Other Symptoms of Varroa ☐ DWV ☐ Mites on adult bees ☐ Black Queen Cells ☐ Varroa  
fecal matter

Have any other disease/pests problems been noted in the colonies? ☐ Yes ☐ No

Check all that apply: ☐ Wax moths ☐ Small hive beetle ☐ AFB ☐ EFB ☐ Chalkbrood ☐  
Diarrhea/Nosema

Were any of the following present: ☐ Swarm Cells ☐ Supersedure Cells ☐ Excessive Drone Combs

Was Robbing Behavior Observed ☐ Yes ☐ No

Condition of Apiary: \_\_\_\_\_

List any blooming plants within 1-2 miles of apiary/distance and direction. Also note when they were in bloom

\_\_\_\_\_

Additional Comments:

\_\_\_\_\_

**Supplement 2.4 Winter 2018 Follow up questions:**

- 1) After completing the survey have you since done any additional colony checks, treatments, or feedings?
- 2) How many colonies are you over wintering? Have you lost any colonies since the survey?

**Supplement 2.5. Spring 2019 Follow Up Questions:**

- 1) How many total colonies are you starting with for the 2019 season?
- 2) How many colonies were successfully overwintered from the previous season?
- 3) How many colonies are being newly introduced this year and what is the source of the new bees (package, nuc, swarm, split, etc.)
- 4) How many of last year's colonies did you lose from winter 2018 until now spring 2019?

## **Supplement 2.6. Spring 2020 Follow Up Questions**

- 1) Have you done any additional colony checks, treatments, or feedings after the summer 2019 beekeeping survey/season?
- 2) How many colonies did you have going into winter?
- 3) How many colonies did you successfully overwinter?
- 4) How many of last year's colonies did you lose from winter 2019 until now (spring 2020)?
- 5) How many total colonies are you starting with for the 2020 season?
- 6) How many colonies are being newly introduced this year and what is the source of the new bees (package, nuc, swarm, split, etc.)



### Supplement 3: Procedures for Sampling from Hobbyist Beekeepers.

#### Sampling Procedure:

Make sure equipment is clean before proceeding with sampling. (See cleaning procedures below.)

Sample up to two hives per apiary. Repeat this protocol for all apiaries surveyed.

1. Select hives randomly.
2. Open hives with assistance of the bee keeper.
3. Look for brood and sample about two square inches of brood comb. Taking brood which looks ill or diseased when possible. (Brown or discolored larvae or capped brood with pin holes.) Cut out a two square inch of comb, if the foundation is too tough to cut gently scrape the comb off the foundation.
4. If there is no brood in a hive, open another randomly selected hive and look for brood. If no brood can be found, sample comb which contained brood. (The darker the comb the more brood has been reared in it.)
5. Place the comb sample in a regular size lunch bag. Sample both hives into the same bag.
6. Once the comb has been sampled take a sample of live bees for the same hive as the comb was taken from.
7. First make sure the queen is not present on the frame to be sampled.
8. Shake frame into a pan and scoop 1/2 cup of bees to be transferred into a sample jar about half full of 70% alcohol. For a composite sample from two hives use ¼ cup from each hive
9. Do this with a frame from the second hive sampled.
10. Once all bees are dead and thoroughly soaked in alcohol, drain off the excess alcohol and discard. (Do not reuse the drained alcohol).
11. Assign a sample number based on the bee keepers last name and the year the sample was taken. I.e.: Jones 2018.
12. Place the jar and bag into a large lunch bag and attach a copy of the sampling form.
13. Mail sample to:

Bee Disease Diagnosis  
Bee Research Laboratory  
10300 Baltimore Ave. BARC-East  
Bldg. 306 Room 316  
Beltsville Agricultural Research Center - East  
Beltsville, MD 20705

Once the sampling is completed, clean the bee equipment.

1. Wash gloves, hive tool and anything else used in the hive in very hot water with soap.
2. Clean the gloves and other equipment with alcohol.
3. Keep clean until the next hive, for instance in a new ziplock bag.

## Supplement 4: Varroa mite sampling protocol

### **Collecting bees:**

Collect a lightly packed 1/2 cup sample (~300) of adult bees (avoiding the queen) between the two hives, collecting approximately 150 bees from each. Transfer adult bees directly into the collection jar from a brood frame by moving collection jar downward over adult bees so they fall backwards. Or shake bees directly from two or three brood frames into a larger collecting container (honey bucket, cardboard container, or lipped tray) and scoop up 1/2 cup of bees and quickly pour them into a quart mason jar. For a composite sample from two hives take a quarter cup from each hive for a total of ~300 bees.

### **Alcohol wash method (You can use soap water/anti-freeze instead)**

Perform the alcohol or soap wash away from the hive.

1. Add enough alcohol (inexpensive rubbing alcohol works well) or soap (use a low-sudsing soap, such as automotive windshield washer fluid) to completely cover the bee sample in the jar.
2. Vigorously shake the jar for at least one minute to dislodge the mites from the bees. To improve the consistency of mite counts, shake the jar for a consistent length of time for every sample.
3. After shaking, empty the liquid contents into a clear plate or white shallow pan through a mesh screen that traps the adult worker bodies.
4. Add more alcohol or soap to the jar and repeat steps 2 and 3 to increase the accuracy of the counts.
5. Count the number of mites in the plate or pan.

### **Counting the mites:**

The goal of mite assessment is to determine the number of Varroa mites per 100 adult bees, expressed as the percentage of infestation.

**Steps:**

1. Count the number of mites collected in the plate or pan.
2. Divide that number by the number of bees in the sample.
3. Multiply by 100 to yield a percentage.

**Example:**

A beekeeper samples 300 adult bees and counts 12 mites in the pan.

$12 \text{ mites} \div 300 \text{ bees} = .04 \times 100 = 4\%$  (4 mites per 100 adult bees)

## Supplement 5: Email-Consent Form

### **Honey Bee Health Mentoring - 19-9143H Approved By Colorado State University's Institutional Review Board 2019**

Dear Participant,

My name is Colton O'Brien and I am a researcher from Colorado State University in the Bioagricultural Sciences & Pest Management department. We are conducting a research study titled Honey Bee Health Mentoring Program, looking at honey bee health mentoring for hobby beekeepers and are reaching out to you based on your previous interest in participating. The Principal Investigator is Kurt Jones in the Horticulture and Landscape Architecture department and I am the Co-PI.

As part of this study, we are asking you to participate in a hive inspection (2-3 hours) and take a brief questionnaire (10 mins). Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participation at any time without penalty.

The researchers are the only people who will have access to your identifiable data and you will not be identified in any publications. As part of the research analysis we will create a map of Colorado that shows where hobbyists were surveyed and will roughly show the GPS coordinates. This map will not include any personal information, just points. While there are no known risks or direct benefits to you, we hope to gain more knowledge on honey bee health mentoring.

If you have questions, would still like to participate or have changed your mind about participating, please contact me at [colton.obrien@rams.colostate.edu](mailto:colton.obrien@rams.colostate.edu). If you have any

questions about your rights as a volunteer in this research, contact the CSU IRB

at: [RICRO\\_IRB@mail.colostate.edu](mailto:RICRO_IRB@mail.colostate.edu); 970-491-1553.

Sincerely,

Colton O'Brien

Kurt Jones